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TRYPANOSOMA BRUCEI AS A FILTERABLE VIRUS

STUDIES ON THE SITE OF ORIGIN OF THE FILTER-PASSING BODIES

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In a recent publication (1922), the existence of filterable bodies in organ macerations derived from animals infected with *Trypanosoma brucei* was reported. In this preliminary communication, a brief review of the literature was included; also, attention was drawn to the technical difficulties involved in filtration experiments, while caution was enjoined in the matter of drawing sweeping conclusions. Using bacteriologically sterile filtrates of organ macerations, infection was induced in normal guinea pigs in ten out of nineteen cases. Inasmuch as the literature on this subject is quite contradictory, it seemed desirable to continue and extend the investigation to include more critical inquiry into the site of the filterable bodies. The protocols which form a portion of this report include the details of the earlier experiments as well as those more directly concerned with the present investigation. The modern developments in the study of the filterable viruses have shed much light on the problem; incidentally, in the mass of data relating to disease entities covering a large biological horizon, it becomes increasingly difficult for one to correlate and interpret experimental findings in accordance with the multiplicity of possibilities that present themselves. The recent review by Simon (1923) achieves much in the direction of orientation for future research; it is felt particularly that this writer's concept of the individuality of research with the filterable viruses constitutes a valid contention. Bearing in mind the many possibilities of error, attention should first be drawn to the question of standardized techniques. With such procedures well in hand, and with controls paralleling every experimental entity, it would seem better to leave final interpretation from the biological outlook, to future research. There is no doubt that ideas relating to the nature of filterable agents of disease are in a stage of transition. Today, no valid definition for the filterable viruses exists. To the writer this seems a happy circumstance, since only too often the rigidity of arbitrary biological bounds has served to hamper or at

least to bias the broader outlook which is the more modern tendency. While admitting the great value of classification, it is felt that too much stress is often laid on taxonomy; fewer polemics of an academic nature would arise if investigators were to content themselves primarily with accumulating accurate, concordant data, on the basis of which an abiding and serviceable nomenclature could be established.

The preliminary experiments demonstrated that organ macerations derived from guinea pigs dead of infection with *T. brucei* would, under appropriate conditions, yield bacteriologically sterile filtrates still capable of inducing infection in normal animals. The present report details the results of the experiments on organ filtrates, in which it was the purpose to determine the site or sites of the filterable forms. This problem was attacked by investigating the infectivity of filtrates of groups of organs, and finally of individual organs. This indirect method was adopted because of the difficulty of handling rapidly and satisfactorily a large number of filtrations at one time; although somewhat involved, it has seemed to subserve the purpose of the investigation.

As in the first experiments, filtration was accomplished by suction. The material to be filtered was macerated and suspended in sterile physiological saline. Following this, the coarser particles were removed by filtration through three layers of sterile gauze; the filtrate from this process being caught directly into the aluminum receptacle enclosing the filter candle. Mandler filters of diatomaceous earth, measuring $2\frac{1}{2} \times \frac{5}{8}$ inch were used. To the menstruum to be filtered, a loopful of a 24 — 48 slant culture of *Bacillus prodigiosus* was added to serve as a bacteriological control for the efficiency of the filter. Immediately following the filtration process, a guinea pig was inoculated intraperitoneally with a quantity of the filtrate, and simultaneously a control animal was inoculated in the same manner with the unfiltered suspension. Control animals were not run in all of the series since a factor of economy is involved and such controls did not seem to be consistently necessary in as much as this was not a statistical study. Immediately following injections, three 2 mm. loopfuls of the filtrate were placed on glucose agar to determine the absence of *B. prodigiosus* while at the same time one loopful of the original maceration was placed on another similar slant. In certain of the earlier experiments the filtration was accomplished at somewhat elevated temperatures, maintained by means of an improvised water bath surrounding the filtering apparatus. The procedure was cumbersome and following the first success with material filtered at room temperature, the use of a higher temperature was abandoned.

In addition to organ filtration, a number of experiments were performed using the heart's blood of infected guinea pigs during the height of the infection. The exact details for this series (which

included seven animals) are not available since the records were lost in a recent fire. However, the blood was diluted with twice its volume of citrated saline and filtered through Mandler filters using *B. prodigiosus* as the bacteriological control. The filtrate was inoculated into guinea pigs. None of the animals developed infection. The animals used for pathogenicity tests were normal stock guinea pigs which had been raised in the department animal house. They were between two and three months of age at the time. The very fact that such animals had been in stock for this period would seem to preclude any possibility of infection with pathogenic trypanosomes. However, examination of the peripheral blood of each animal used was adopted as a routine procedure. As was expected, such examination always proved to be negative.

Further proof of the freedom of spontaneous trypanosome infection in the guinea pigs used was sought by cultural methods, using the following procedure:

Experiment 1.—September 20, 1923: Fifty stock guinea pigs were bled directly from the heart (under partial ether anesthesia), using a 5 c.c. Luer syringe bearing a No. 27 needle. Two or three c.c. were thus obtained from each animal. The blood was disposed of as follows:

1. The blood from 25 animals was injected directly into a flask containing 50 c.c. of sterile 1 per cent. sodium citrate in physiological saline.

2. The blood from each of the remaining twenty-five was inoculated directly into tubes of NNN medium (containing three volumes of defibrinated rabbit's blood).

At this time, six tubes of NNN medium were inoculated with a drop each of blood derived from a guinea pig which had been experimentally infected. Also 2 c.c. of infected blood were inoculated into a flask containing 50 c.c. of sterile sodium citrate saline. These controls, together with the blood from normal animals, were incubated at room temperature, and examined daily for parasites. In these examinations, both fresh and stained smears were utilized.

Although examination of the cultures was continued over a period of three weeks, nothing in the nature of trypanosomes was ever encountered in the material derived from stock guinea pigs. On the other hand, both the saline and NNN cultures of infected blood showed multiple rosette formation and contained active parasites during the time of investigation.

Experiment 2.—Twenty stock guinea pigs were bled from the heart and the blood collected into 50 c.c. of sterile sodium citrate in saline. The material was divided between two large tubes and centrifugalized at high speed for five minutes. The supernatant fluid was pipetted off, and the residue in each tube taken up in 2 c.c. of saline. With this material four white rats were inoculated intraperitoneally with 0.5 c.c. each of the saline suspension.

At the end of six weeks observation, these animals are alive and normal.

Experiment 3.—Thirty guinea pigs were bled from the heart. The blood was allowed to clot at ice box temperature for six hours. The serum was then pipetted off, and with it four white rats were injected intraperitoneally with 2 c.c. of serum each. (It is a common observation that if infected blood be allowed to clot, the majority of trypanosomes are found to migrate into the serum).

None of these animals developed infection.

PROTOCOL No. 1.—*Filtrates of Combined Liver, Spleen, Lungs, Bone-Marrow, Inguinal Lymph Glands, and Heart's Blood*

Experiment†	Date Commenced	Animal Number	Remarks on Microscopic Findings in Organs Filtered	Diluent, C.c.	Vacuum, Inches of Mercury	Time. Minutes	Temperature	Amount Injected, C.c.	Culture of Maceration	Culture of Filtrate	Results	Controls
I	2/23/22	530A*	Much nuclear detritus, intracorpuscular forms in the spleen and lungs (?)	20	20	30	RT	2.0	+	—	3/ 8/22, positive light infection 3/16/22, crises (2,000,000 mm.) 3/19/22, dead	3/ 8/22, positive 3/20/22, dead streptococcus peritonitis
II	2/24/22	530	None..................	20	25	25	RT	1.0	+	—	3/ 8/22, negative 3/16/22, negative 3/25/22, negative	3/ 7/22, positive 3/ 8/22, crisis 3/16/22, dead
III	2/24/22	43	Rounded flagellate forms in liver and spleen (2-3 μ diameter) scattered nuclear masses	20	20	45	RT	2.0	+	—	2/28/22, eaten by rats	2/31/22, positive 3/ 8/22, dead
IV	2/24/22	430‡	None..................	20	20	30	45°C.	2.0	+	+	2/27/22, negative 3/ 1/22, positive	2/27/22, positive 3/21/22, dead
V	3/21/22	546*	None..................	20	30	60	45°C.	3.0	+	—	3/25/22, positive 4/ 2/22, dead	None
VI	3/28/22	532*	Fusiform types in liver and spleen; no flagellum or organelle	20	22.5	30	RT	3.0	+	—	4/ 2/22, negative 4/ 6/22, negative 5/20/22, positive (delayed infection)	4/ 2/22, negative 4/ 6/22, negative 4/ 8/22, positive 4/16/22, dead
VII	3/29/22	584‡	Fusiform types in liver; nuclear masses in spleen; bone-marrow shows much destruction	20	22	30	RT	1.5	+	+	4/ 5/22, positive	4/ 5/22, positive
VIII	3/30/22	583	Large and small races in liver, spleen and heart's blood; small forms show no membrane or flagellum; dividing forms in blood	20	20	60	RT	2.0	+	—	3/31/22, negative 4/ 5/22, negative 5/20/22, negative 7/14/22, dead balantidium infection	4/ 5/22, negative 4/ 6/22, positive 4/16/22, dead
IX	4/ 5/22	522*	Rounded form in liver and spleen clusters with centrifugal flagella	20	20	30	RT	2.0	+	—	4/ 8/22, negative 4/14/22, positive	None

X	4/17/22	538*	Rounded type in spleen; nuclear mass appearances	20	21	60	RT	1.5	+	—	5/ 1/22, positive heavy infection 5/ 4/22, dead	4/19/22, positive 4/26/22, dead
XI	4/27/22	567*	None....................	20	15	60	RT	1.5	+	—	4/29/22, negative 5/ 6/22, negative 5/18/22, negative	4/29/22, negative 5/ 6/22, positive
XII	5/18/22	527	Numerous typical parasites in liver, spleen, heart's blood and lungs	20	20	45	RT	2.0	+	—	5/19/22, negative 5/22/22, negative 5/31/22, negative 6/ 8/22, negative	5/19/22, negative 5/22/22, positive 6/ 1/22, dead
XIII	5/20/22	387‡	None....................	20	20	40	RT	2.0	+	+	Discontinued	Discontinued
XIV	5/24/22	262‡	Nuclear masses in spleen, bone-marrow; intracorpuscular forms in lungs (?)	20	22.5	45	RT	1.5	+	+	5/25/22, negative 5/28/22, positive Discontinued	Discontinued
XV	5/24/22	274‡	Numerous trypanosomes in liver and spleen	20	30	45	RT	2.0	+	+	5/28/22, negative Discontinued	Discontinued
XVI	5/25/22	268	Rounded flagellate and plasmodial types in spleen; minute fusiform organisms in liver	20	25	60	RT	1.5	+	—	5/28/22, negative 6/ 6/22, negative 6/12/22, negative 6/18/22, negative	5/28/22, positive 6/ 4/22, dead
XVII	6/ 8/22	341*	Chloroformed at crisis; multiple stages in liver and spleen	20	20	45	RT	1.5	+	—	6/12/22, negative 6/30/22, dead (trypanosomes in liver, spleen and round forms in blood	None
XVIII	6/16/22	68*	Nuclear fragments in spleen, nodes and bone-marrow; rounded forms in liver	20	15	40	RT	1.0	+	—	6/19/22, negative 6/22/22, negative 6/28/22, positive	6/19/22, negative 6/22/22, positive crisis
XIX	9/ 1/22	150	None....................	20	15	45	RT	1.5	+	—	9/10/22, negative 9/15/22, negative 9/22/22, negative	9/10/22, negative 9/15/22, negative 9/22/22, negative
XX	1/ 9/23	4*	Rounded flagellate bodies in liver and spleen, nuclear detritus in bone-marrow	10	26	60	RT	1.5	+	—	1/11/23, negative 1/16/23, negative 1/18/23, positive 1/23/23, dead	1/11/23, positive 1/15/23, dead streptococcus peritonitis
XXI	2/ 2/23	18	Nuclear particles in bone-marrow	10	25	45	RT	1.0	+	—	2/ 6/23, negative 2/18/23, negative 2/26/23, negative 3/ 3/23, negative	None

† Records of approximately ten other experiments in this series were lost in a recent fire. * Positive results. ‡ Defective filters.

Protocol No. 2.—*Fractional Filtrates: 1, Liver and Spleen; 2, Bone-Marrow and Lymph Nodes; 3, Heart and Lungs*

Experiment	Date Commenced	Animal Number	Organs Filtered	Remarks on Microscopic Findings in Organs	Diluent, C.c.	Vacuum, Inches of Mercury	Time in Minutes	Temperature	Amount Injected, C.c.	Culture of Maceration	Culture of Filtrate	Results	Controls
I	3/21/22	521	L & S	None....................	20	30	20	RT	3	+	—	3/25/22, negative 3/26/22, negative 3/30/22, negative 4/ 7/22, negative Discontinued	4/ 7/22, negative
		548	B & Ly	None....................	20	20	20	RT	1	+	—	3/25/22, negative 3/26/22, negative 3/30/22, negative 4/ 7/22, negative Discontinued	4/ 7/22, negative
		549*	H & Lu	Intracarpuscular forms in lung smear ?	20	30	30	RT	1.5	+	—	3/25/22, negative 3/26/22, negative 3/30/22, negative 4/ 7/22, dead Liver and spleen show parasites in various stages	3/30/22, positive
II	5/18/22	215‡	L & S	None....................	20	20	30	RT	1.5	+	+	5/23/22, positive	5/23/22, positive
		387‡	B & Ly	Nuclear masses in bone-marrow	20	25	45	RT	2	+	+	5/23/22, negative 5/26/22, negative 5/30/22, negative Discontinued	5/23/22, negative
		388	H & Lu	None....................	20	20	30	RT	0.5	+	—	5/20/22, negative 5/25/22, negative 6/ 1/22, negative	5/23/22, positive
III	9/ 1/22	627*	L & S	Nuclear masses faint elongated parasites	20	15	60	RT	1.5	+	—	9/15/22, positive 9/17/22, heavy infection 9/20/22, died	9/15/22, positive 9/17/22, dead
		390	B & Ly	None....................	15	15	60	RT	1.5	+	—	9/15/22, negative 9/22/22, negative	9/22/22, negative
		650	H & Lu	None....................	15	15	60	RT	1.5	+	—	9/15/22, negative 9/22/22, negative	9/15/22, positive 9/27/22, dead
IV	9/28/22	872*	L & S	Nuclear detritus in liver and spleen, no organized bodies	20	20	30	RT	2.0	+	—	10/ 1/22, negative 10/11/22, positive 10/28/22, dead	10/11/22, positive 10/30/22, dead (Note that control lived longer)
		871	B & Ly	None....................	20	20	30	RT	1.0	+	—	10/ 1/22, negative 10/11/22, negative 10/12/22, negative 10/28/22, negative	10/28/22, negative
		889	H & Lu	None....................	20	15	30	RT	0.5	+	—	10/ 1/22, negative 10/11/22, negative 10/20/22, negative 10/28/22, negative	10/11/22, positive 10/20/22, dead

V	11/ 9/22	149	L & S	None	20	30	45	RT	0.5	+	—	11/12/22, negative 11/29/22, negative 1/12/23, negative 1/14/23, dead (injected normal animal with organ emulsion, no inf.)	None
		149A	B & Ly	None	20	30	40	RT	1.0	+	—	11/22/22, negative 11/29/22, negative 1/12/23, negative	None
		149B	H & Lu	None	20	25	30	RT	1.0	+	—	11/12/22, negative 11/29/22, negative 1/12/23, negative	None
VI	1/ 3/23	171*	L & S	Nuclear fragments	10	20	30	RT	1.5	+	—	1/ 5/23, negative 1/ 9/23, positive 1/16/23, heavily infected	1/ 9/23, positive
		172	B & Ly	None	10	22.5	30	RT	1.5	+	—	1/ 5/23, negative 1/ 9/23, negative 1/16/23, negative	1/ 9/23, negative 1/16/23, positive
		180	H & Lu	None	10	20	30	RT	1.5	+	—	1/ 5/23, negative 1/ 9/23, negative 1/16/23, negative	1/ 9/23, negative 1/16/23, negative
VII	1/ 7/23	118	L & S	None	10	15	45	RT	1.5	+	—	1/ 9/23, negative 1/16/23, negative 1/27/23, negative	1/16/23, positive 1/31/23, dead
		119	B & Ly	None	10	15	60	RT	1.5	+	—	1/ 9/23, negative 1/16/23, negative 1/27/23, negative	1/27/23, negative 1/31/23, negative
		120	H & Lu	None	10	15	60	RT	1.5	+	—	1/ 9/23, negative 1/16/23, negative 1/27/23, negative	1/27/23, negative 1/31/23, negative
VIII	2/ 6/23	393*	L & S	Nuclear masses in liver and spleen; flagellated oval bodies	10	20	45	RT	1.5	+	—	2/ 9/23, negative 2/10/23, negative 2/14/23, positive 2/18/23, highly inf. 2/28/23, dead	None
		394	B & Ly	Nuclear detritus in bone-marrow	10	20	45	RT	1.5	+	—	2/ 9/23, negative 2/10/23, negative 2/14/23, negative 2/18/23, negative 2/28/23, negative	None
		395	H & Lu	None	10	20	45	RT	1.5	+	—	2/ 9/23, negative 2/10/23, negative 2/14/23, negative 2/18/23, negative 2/28/23, negative	None
IX	2/28/23	28	L & S	Minute fusiform parasites in liver and spleen; membrane and flagellum not discernible	10	15	30	RT	1 5	+	—	3/ 4/23, negative 3/10/23, negative 3/20/23, negative	None
		29	B & Ly	None	10	15	30	RT	1.5	+	—	3/ 4/23, negative 3/10/23, negative 3/20/23, negative	None
		30	H & Lu	None	10	22	30	RT	1.0	+	—	3/ 4/23, negative 3/10/23, negative 3/20/23, negative	None

Protocol No. 2.—*Fractional Filtrates: 1, Liver and Spleen; 2, Bone-Marrow and Lymph Nodes; 3, Heart and Lungs—(Continued)*

Experiment	Date Commenced	Animal Number	Organs Filtered	Remarks on Microscopic Findings in Organs	Diluent, C.c.	Vacuum, Inches of Mercury	Time in Minutes	Temperature	Amount Injected, C.c.	Culture of Maceration	Culture of Filtrate	Results	Controls
X	4/ 1/23	31*	L & S	None....................	10	20	30	RT	1.5	+	—	4/ 3/23, negative 5/ 8/23, positive 4/18/23, dead	None
		32	B & Ly	None....................	10	20	30	RT	1.5	+	—	4/ 3/23, negative 4/18/23, negative 5/ 1/23, negative	None
		33	H & Lu	None....................	10	20	30	RT	1.5	+	—	4/ 3/23, negative 4/18/23, negative 5/ 1/23, negative	None
XI	4/ 2/23	34*	L & S	Leishmania and herpetomonad types in liver and spleen	10	20	30	RT	1.5	+	—	4/25/23, negative 5/ 6/23, positive 5/ 9/23, positive 5/11/23, dead	None
		35*	B & Ly	None....................	10	20	30	RT	1.5	+	—	4/25/23, negative 5/ 6/23, positive 5/ 9/23, positive 5/15/23, dead	None
		36	H & Lu	None....................	10	20	30	RT	1.5	+	—	4/25/23, negative 5/ 6/23, negative 5/11/23, negative 5/15/23, negative	None
XII	5/ 3/23	47	L & S	None....................	10	15	45	RT	1.5	+	—	5/ 7/23, negative 5/17/23, negative 5/26/23, negative 6/ 3/23, negative	None
		38	B & Ly	None....................	10	17	45	RT	1.5	+	—	5/ 7/23, negative 5/17/23, negative 5/26/23, negative 6/ 3/23, negative	None
		39	H & Lu	None....................	10	15	45	RT	1.5	+	—	5/ 7/23, negative 5/17/23, negative 5/26/23, negative 6/ 3/23, negative	None
XIII	6/10/23	43*	L & S	None....................	15	15	40	RT	1.0	+	—	6/13/23, negative 6/18/23, positive 6/22/23, positive 6/31/23, dead	None
		45	B & Ly	None....................	15	15	40	RT	1.0	+	—	6/13/23, negative 6/18/23, negative 6/22/23, negative 6/31/23, negative	None
		46	H & Lu	None....................	15	15	40	RT	1.0	+	—	6/13/23, negative 6/18/23, negative 6/22/23, negative 6/31/23, negative	None

* Positive infections. ‡ Defective filters.

PROTOCOL NO. 3.—*Individual Filtrates of (1) Liver, (2) Spleen*

Experiment	Date Commenced	Animal Number	Organ Filtered	Remarks on Microscopic Appearance of Source	Diluent, C.c.	Vacuum, Inches of Mercury	Time in Minutes	Temperature	Amount Injected, C.c.	Culture of Maceration	Culture of Filtrate	Results	Controls
I	5/ 1/23	51*	Liver	Amoeboid clusters with centrifugal flagella; also slender forms of small size	10	20	30	RT	1.5	+	—	5/ 3/23, negative 5/ 8/23, negative 5/11/23, positive 5/20/23, dead	5/ 3/23, negative 5/ 8/23, positive
		52	Spleen	Leishmania-like bodies; nuclear bodies	10	22.5	30	RT	1.5	+	—	5/ 3/23, negative 5/ 8/23, negative 5/11/23, negative 5/20/23, negative 6/ 1/23, negative	5/ 3/23, negative 5/ 8/23, positive
II	5/ 3/23	53	Liver	None....................	10	15	45	40°C.	1.5	+	—	5/ 8/23, negative 5/11/23, negative 5/20/23, negative 6/ 1/23, negative	5/ 8/23, negative 5/11/23, negative 6/ 1/23, negative
		54	Spleen	None....................	10	15	45	40°C.	1.5	+	—	5/ 8/23, negative 5/11/23, negative 5/20/23, negative 6/ 1/23, negative	5/ 8/23, positive
III	5/11/23	55	Liver	Few typical trypanosomes	10	20	30	RT	1.5	+	—	5/22/23, negative 5/31/23, negative 7/ 8/23, negative	5/22/23, positive
		56*	Spleen	Nuclear masses, free flagella with attached centrosome	10	20	30	RT	1.5	+	—	5/22/23, negative 5/31/23, negative 7/ 8/23, negative	5/22/23, positive
IV	5/28/23	57	Liver	Plasmodial groups (syncitia), very faint cytoplasm	10	25	30	RT	1.0	+	—	6/ 3/23, negative 6/ 6/23, negative 7/ 8/23, negative	6/ 3/23, negative 6/ 6/23, positive
		58	Spleen	Similar to 57	10	25	30	RT	1.0	+	—	6/ 3/23, negative 6/ 6/23, negative 7/ 8/23, negative	6/ 3/23, negative 6/ 6/23, negative 7/ 8/23, negative
V	7/ 8/23	59	Liver	None....................	10	25	45	RT	1.5	+	—	7/15/23, negative 7/22/23, negative 7/30/23, negative	7/15/23, negative 7/22/23, positive
		60	Spleen	Nuclear detritius.........	10	15	45	RT	1.5	+	—	7/15/23, negative 7/22/23, negative 7/30/23, negative	7/15/23, negative 7/22/23, dead
VI	7/14/23	61	Liver	None....................	10	20						7/22/23, negative 7/31/23, negative 8/ 3/23, negative 8/11/23, negative	7/22/23, positive
		62	Spleen	None....................	10	23						7/22/23, negative 7/31/23, negative 8/ 3/23, negative 8/11/23, negative	7/22/23, positive

* Positive infection.

The fact that all of the cultures using blood from normal guinea pigs yielded negative results, whereas the method used will reveal parasites when infected blood is used, lends further proof of the absence of spontaneous infection in guinea pigs. Furthermore, in spite of the thousands of times in which guinea pigs have served as experimental animals for workers all over the world, there is no record of encountering pathogenic trypanosomes in these animals.

The details for each experiment on organ filtrates together with the particular result are exhibited in the protocols which form a part of this report.

DISCUSSION

The positive results obtained in this investigation indicate that *Trypanosoma brucei* undergoes certain changes in morphology in the internal organs of such nature as to permit passage through the pores of bacteria proof filters. It would seem that such forms reside in greatest numbers in the liver and spleen of infected animals, although the lungs, bone-marrow, heart's blood and lymphatic glands may occasionally harbor filterable bodies. Experiments with highly infected peripheral blood, obtained during the life of the animal, indicate the absence of filterable stages in the circulation. However, more extended studies in this direction, using peripheral blood during successive stages of the infection, would be of interest.

These studies are not statistical in their nature; they are qualitative rather than quantitative. It is quite conceivable and indeed probable that an extension or even a duplication of these experiments might yield results which from a quantitative standpoint would be at variance with the recorded findings. Differences in technique, peculiarities of the strain, or specific transformations in morphology correlated with definite phases in the life cycle are some of the important points which undoubtedly play a determinative rôle in such an investigation.

The nature of the filter passer cannot be predicted from these experiments. The possibilities include (1) plasticity of the trypanosomes, (2) slender forms arising out of repeated and rapid division, (3) intracellular or extracellular bodies of variable size. In connection with the second possibility, one recalls Schaudinn's (1904) early observations on the minute asexual forms of trypanosomes found in the gut of the mosquito. Although this writer did not attempt experimental confirmation, he was led to predict the possible filterability of these small bodies. The more recent observations of McCullough (1919) on endogenous budding in *Crithidia euryopthalmi* (a flagellate parasite of the lupine bug) indicates changes of this nature as a possible source of the filterable entities.

It is felt that extensive filtration studies on protozoa in general will bring about modifications in our concepts regarding the interrelationships which exist between these microörganisms on the one hand, and such poorly defined genera as Chlamydozoa, Strongyloplasma, and

Spirochaeta on the other hand. At any rate, a more precise biological definition for the filterable viruses should take the place of the vague generalizations now found in the literature.

CONCLUSIONS

1. In a series of seventy-two filtration experiments, using the organs of guinea pigs dead of infection with *Trypanosoma brucei,* inoculation of bacteria-free filtrates into normal guinea pigs has determined infection in twenty cases.

2. It would seem that the filterable forms reside in greatest number in the liver and spleen of guinea pigs dead of infection.

3. In a series of seven experiments, the filtrates from highly infected blood obtained during the life of the animal did not prove infective for guinea pigs.

4. Stock guinea pigs do not harbor trypanosomes.

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SOME ANCIENT WORTHIES IN PARASITOLOGY

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FRANCESCO REDI

Like de Geer, Francesco Redi (1626-1698) was born of a patrician family and like Loewenhoek he did not share the poverty of Linnaeus. He was born at Arezzo where his tomb still attracts many visitors. He died at Pisa where he had studied. He was, as people in his age were, curiously versatile.

He was a courtier, a scholar of distinction, and took a prominent part in preparing the Dictionary de la Crusca which was being issued in 1691. He was physician to the Dukes of Tuscany, including Cosimo III (Swammerdam's Duke). He was a poet whose sonnets are still read, but his chief work as a writer of verse was his *Bacco in Toscano, Ditirambo con annotazioni,* in which he wrote rapturously in favor of wine, though tradition tells us he never drank it. He was the only teetotaller I have ever come across who adopted such a course:

Even Redi, though he chaunted
 Bacchus in the Tuscan valleys,
Never drank the wine he vaunted
 In his dithyrambic sallies.

—*Longfellow*

He was, further, a great man of science and a most brilliant investigator. Amongst his earliest experiments was a research on the poison of vipers and he describes how one can swallow their venom without ill effect. Only when the poison is applied to a wound does suffering, and sometimes death, follow.

Redi was amongst the first to attack the doctrine of abiogenesis or spontaneous generation, which, from the time of Aristotle onwards, had held the field. This doctrine maintained that insects, worms and other animals were directly generated in mud, putrefying flesh, etc., and need have no living progenitors. His experiments proved for the first time that such organisms only arose from the egg of the mother fertilized by the spermatozoa of the father. At the time of his writing this was a great advance, for even at the present time there are people of repute who hold that bacteria and other microscopic organisms are generated without an antecedent parent. He made a most important series of experiments on the generation of insects. His experiments demonstrated that more than one kind of fly can come from the flesh of one kind of

animal, whilst different kinds of flesh yielded the same fly. He investigated the egg-laying of flies, dissected their ovaries and proved that the appearance of a particular species of insect in flesh depended entirely on the kind of insect which had deposited the ova. He discovered that no flies originated from flesh that was kept in closed bottles whilst the flesh inside open receptacles gave rise to them. Of course he made mistakes. He at one time thought that grubs inside nuts or fruits were produced by the same "virtue" which led to the production of the particular part of the plant infected.

He investigated the action of the stones in the gizzards of birds and he also added something to our knowledge of the life-history of the eel. He showed in his *Osservazioni intorno agli animali viventi che se trovano negli animali viventi* (Firenze 1684) that parasites were parasites, and not parts of the host. He also investigated the cestodes of marine fishes and suggested the use of mercury as an helminthic. In many respects he may be regarded as the father of Helminthology.

He was certainly the first investigator to investigate the Helminthes with success. He investigated the tape-worms of cats and dogs and recognized at any rate in part the species *Taenia crassicollis* and its proglottides. He investigated the *Cysticercus pisiformis* from the mesentery of the hare and determined that the fluid within the "bladder-worm" did not coagulate by boiling. He described the cysticercus forms as "glandulae" or "bullae" or "hydatides pellucidae cum acumine candido non pellucido." He also recognized the head with four points (suckers). On the whole he made very distinct advances in the Science of Zoology and was highly thought of by his contemporaries and successors, such as Swammerdam, Loewenhoek and Ray.

CAROLUS LINNAEUS

Carolus Linnaeus (1707-1778) was the eldest child of Nils Linnaeus and of Christina daughter of Brodersonius. The father had succeeded his father-in-law as pastor of the parish of Stenbrohult and Linnaeus was emphatically a son of the manse. Unlike de Geer his life for many years was a constant struggle against poverty. His very early childhood was marked by a distinct inclination for the study of natural history. He was for three years at the gymnasium at Wexiö. Here, during the first two years he made so little advance in his studies that his father had decided to apprentice him to a tailor or shoemaker, and this would probably have been done had not a Dr. Rothman offered to board and lodge Carl for the remaining twelve months of his course and to instruct him in physiology.

At the University of Lund where he proceeded in 1727 he found another friend in Dr. Stobaeus, Professor of Medicine, whose library

and collection of rareties were, after a time, freely placed at the disposal of the young naturalist. In 1728 he had left Lund for Upsala, where he suffered great privations owing to want of means; but here again he found a friend in Dr. Olaf Celsius who not only put him up but assisted him to obtain pupils.

It was about this time, one is inclined to think, that he wrote his first dissertation on the sexes of plants. In May, 1732, he started on his well-known journey throughout Lapland, where amidst considerable dangers and hardships he travelled nearly 5000 miles at a cost of less than $125. In 1733 he was again at Upsala teaching mineralogy and hoping to teach botany. But a violent quarrel with a rival, Dr. Rosen, ended in his leaving the University and proceeding abroad with the object of taking a medical degree. Traveling by Lübeck and Hamburg he reached Amsterdam by sea and almost immediately proceeded to Harderwijk where he passed the necessary examinations and defended his thesis on the cause of intermittent fever. At that time the *Systema Naturae* was in manuscript and Grovonius was so delighted at seeing it that he offered to pay the expenses of publication. A wealthy British banker, Cliffort, took Linnaeus to live with him, and here for a time he studied both his patron's magnificent garden and his well supplied library.

In 1736 Linnaeus visited England where he was somewhat coldly received by Sir Hans Sloan in London; but he was welcomed at Oxford. Returning to the Netherlands he published his *Genera Plantarum*, "the starting point of modern systematic botany." At first unknown, he gradually forced his way to the front as a physician and was at last in a position to marry Sara Moré to whom he had been long affianced. In the following year (1740) the chairs of Botany and Medicine at Upsala became vacant and his old enemy, Rosen, and he were chosen respectively to fill them. The two rivals agreed to change professorships and we find Linnaeus established in the position he had so long sought. In 1761 he was granted a patent of nobility and from that time was called Carl von Linné. The arms he chose were those now borne by the Linnaean Society of London.

Linnaeus' great work was that of classifying and systematizing. It has been said of him that "he found biology a chaos, he left it a cosmos." During his life-time owing to numerous explorations and voyages new animals were constantly being recorded. By his system they were readily sorted out. The binomial method of naming animals was his and he was peculiarly gifted in recording the salient features of an organism in the tersest manner. He was also a great teacher and had a magnetic power of impressing himself upon students who came to him from all parts. In the later years of his life Upsala was a center

of the biological sciences and the adulation that was showered upon Linnaeus perhaps explains a certain amount of his conceit and vanity —of which a characteristic sign was replacing his Carolus Linnaeus by Baron Carl von Linné.

CHARLES BARON DE GEER

Charles Baron de Geer (1720-1778) was a member of a wealthy Dutch family long settled in Sweden. Their wealth was derived from certain iron mines and furnaces. He had been destined for a political career; but from a boy he had shown great interest in natural history. At the age of sixteen he had investigated the habits of the water spider and before he was twenty he had published his first paper, *An Account of Podura and Other Spring Tales*. He had been educated in Holland and later attended the lectures of Linnaeus at Upsala. Later he published many other papers, but his name is immortalized by his great work on insects entitled *Mémoires pour servir à l'histoire des Insectes* in seven quarto volumes, Stockholm 1752-1778. He modelled his work on Reaumer's *Histoire des Insectes* and wrote in French, though, as he himself said, his style was "pas trop française." His first volume found but few purchasers, and in a fit of disappointment he destroyed a large part of the edition and ever since then this particular volume has been scarce. This great work included the description of more than 1500 species and was excellently illustrated. His classification was largely based upon the character of the wings and mouth parts, and for the apterous insects on the nature of the metamorphosis. It was an improvement on any classification that had gone before and is still in many respects that which we continue to employ.

As a parasitologist he is perhaps best remembered as having distinguished the two species of body lice. "Il y a donc une différence palpable entre ces deux sortes de Poux, qui semble indiquer qu'ils sont d'espèce différente, à moins qu'on ne veuille plutôt, comme a fait M. de Linné, les regarder comme de deux variétés. Quoiqu'il en soit, on pourrait les distinguer par les dénominations suivantes:

1. Pou de couleur cendrée, à corcelat et ventre bordés d'une raye noire découpée en tâches.

Pediculus (humanus capitis) cinerus, thorace abdomineque facis interrupta nigra marginatis.

2. Pou entièrement d'un blanc sale sans tâches ni rayes. Pediculus (humanus corporis) albidus totus immaculatus."

He is usually regarded as the author of the name *Pediculus capitis* de Geer, 1778. The great attention which was paid to lice during the World War has, however, reversed his opinion and his two species are now treated as one, under the name of *Pediculus humanus*.

De Geer was a public spirited man and devoted part of his great wealth to the relief of others. He re-installed the mines of Danmora which had been flooded and thus enabled a large number of miners to return to work. His talents were recognized by the King who raised him to the dignity of Maréchal de la Cour and gave him the order of Wassa. He was a member of the Academy of Sciences at Stockholm to which he left his great collection of natural history objects that is still preserved in the halls of the Academy at Stockholm.

CHARLES ASMUND RUDOLPHI

The distinguished Swedish naturalist was born at Stockholm, the son of a schoolmaster and pastor who later held a cure near Stralsund in that part of Pomerania which was then part of the Swedish kingdom. He received the ordinary classical education of the day at Greifswald where he took the degree of Ph.D. in 1793. He then turned to medicine and studied at Jena, Dresden, Erlangen and Göttingen. His thesis, which he presented for the degree of Doctor of Medicine at Greifswald, was entitled *sur les vers intestinaux*. In 1779 he became Professor of Medicine in the University, and five years later he visited the chief Universities in Holland, Switzerland, Germany and France. In 1810 he was called to be Professor of Anatomy and Physiology at Berlin. By this time his reputation was well-established and he was appointed Director of the Museum in the Academy of Sciences. Under his influence Berlin became a great center for the study of human and comparative anatomy, both in health and in disease. He exercised a great influence on the medical profession; he had, indeed, a world-wide reputation and was an honorary member of more than 40 learned societies. After him, Wildenow named the well-known leguminous plant, *Rudolphia*.

Rudolphi married twice. First in 1797, with his cousin Friederike Eleonore Wilhelmi, who died in 1801. After his tour abroad he married the eldest daughter of the Burgomaster of Greifswald, Charlotte Friederike Wilhelmie Meyer, in order to give his two daughters—one of whom afterwards married Purkinje—some motherly care. The only son of the second marriage was a doctor. His second wife died in 1821, and after her decease Rudolphi's health began gradually to weaken and he suffered from many of the maladies of premature old age. He was not only an anatomist of distinction but a good authority on mineralogy and a great collector of medals, especially those with portraits of distinguished physicians or men of science. These he left to the Academy. Judging from his portrait he was a well-set-up, comfortable-looking gentleman in prosperous circumstances.

He added materially to our knowledge of parasites. The number of new species which he described is so great that he has been called the father of helminthology and the Linnaeus of parasitic animals. He showed us that trematodes are hermaphrodites and also introduced the name into literature. Zeder in 1800 had grouped animal parasites in five classes: Round-worms, Hook-worms, Sucking-worms, Tape-worms and Bladder-worms. Rudolphi, with some differences, adopted these groups but re-named them Nematoda, Acanthocephala, Trematoda, Cestoidea and Cystica.

Rudolphi described *Strongylus gigas* and he seems to have been the first to see *Taenia echinococcus*. He immensely increased the number of known species. In the thirteenth edition of Linnaeus, edited by Gmelin, the number of parasites had reached 299, and Zeder in 1803 had increased these to 391; but Rudolphi names 1100, taken from 756 hosts.

His principal works are *Observationes circa vermes intestinales,* Greifswald, 1793-5, in two parts; *Entozoorum historia naturalis,* Amsterdam, 1808-10, in two volumes; and *Entozoorum synopsis,* Berlin, 1819. This work, which completes the preceding, contained nine hundred and ninety-three species, of which five hundred and fifty-two are well determined.

DIPHYLLOBOTHRIUM LATUM IN MINNESOTA

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Soon after taking up work in Minnesota I was much impressed by the number of cases of the broad or fish tapeworm (*Diphyllobothrium latum*) in man which are to be met in this section. With most workers in parasitology, I had considered its occurrence as a rarity and as limited almost wholly, if not altogether, to imported cases. While I was acquainted with the work of Nickerson, (1906) I had overlooked his note of 1911 which was a brief paragraph in *Science* in the abstracts of papers presented before the American Association in 1910-1911. Without any special effort to search them out, there have come to my attention in the last five years fourteen cases of the occurrence of this worm in Minnesota alone. Seven of these cases are reported for the first time in my note of 1919. The remaining seven have not been reported.

One of these, which has been brought to my attention by Dr. Macnider Wetherby of St. Mary's Hospital, Duluth, Minnesota, is of special interest because of the question raised as to the length of time that the infection has persisted.

"Mrs. I——— G———, aged 39. Nationality, Jewish. Born in Russia, lived there six years, has been a resident of Superior, Wis., and Duluth, Minn., for the past thirty-three years. Patient entered St. Mary's Hospital, Oct. 9, 1923, for confinement. Given castor oil and quinine that evening. Next morning had an uneventful delivery; a few hours later passed a few feet of segments, and later in the day passed what appeared to be a complete specimen. Specimen estimated eight meters in length. This patient states that she has always been in good health and that prior to passing a few segments in the stool three months ago, has had no sign or symptom of her trouble. Patient states she is extremely fond of fish, especially lake trout.

"Physical examination other than anemic appearance and pasty complexion, is unessential.

"Blood picture: Hbg., 65 per cent., Talqvist; r. b. c., 3,420,000; leuc., 16,200; diff. pmn., 59 per ceit.; lymph., 27 per cent.; eosin, 14 per cent.

Long-lived as this worm is known to be, there can be little question but that the infection in this case was locally acquired. Contrary to earlier evidence it is now clear that such opportunity exists over a considerable area. The first clear-cut evidence that this parasite is endemic in certain sections of this country was afforded by Dr. Nickerson's case of a two-year-old child at Ely, Minnesota. His over-looked case reported in *Science* for 1911 was that of a woman who was born and always lived in Hennepin County, Minnesota, except for one visit to North Dakota where she ate dried and smoked but otherwise uncooked fish. While this makes possible the infection having been acquired in

North Dakota, the probability is that it was acquired in Minnesota. Warthin (1912) records another such case from northern Michigan, while Becker (1916) adds one from Chicago.

In 1919 I reported the case of a young Chippewa Indian child on the White Earth Indian Reservation in Mahnomen County, Minnesota, who had never been off the reservation. I also reported the case of a Minneapolis boy of eight years of age who has lived for all of his life in the State. More recently Calvin (1922) has reported two cases of native-born Jewish children in Chicago. The first of these had eaten of fish only perch, whitefish, and Great Lakes trout. Of the second Calvin states, "The family ate some uncooked smoked fish in the winter. All other fish was cooked (fried or boiled)." Wallace and Green (1922) report a case likewise acquired from eating native fish. The patient was a woman of twenty-five years of age, born in Decatur, Indiana, of German parentage. She lived in Decatur until four and one-half years ago when she moved to Fort Wayne. She had occasionally eaten a little raw fish from Lake Erie. In 1918 she passed about eighteen feet of broad tapeworm and then three sections twelve to eighteen inches in length. In October 1921, on treatment with magnesium sulphate followed by male fern and kamala she passed more than seven feet additional.

Leaving out of consideration the above case of Dr. Wetherby, there are thus known at least nine cases of native infestation with *Diphyllobothrium latum* or a closely related species. I have not made a detailed search of literature and I may have overlooked some other clear-cut case. The greater number of those on record leave open the question of source of infestation, but give evidence of its having been acquired here. It should be borne in mind that, as has been suggested by various writers, the species may not be *Diphyllobothrium latum,* but a closely related form found in our native carnivores.

As to the prevalence of the Diphyllobothrium in Minnesota, Nickerson's and my records, without making any pretense at completeness, total sixty-five for this state alone. This total is out of all proportion to the cases of Taenia which are to be seen in this section. My earlier impression that the Diphyllobothrium is in Minnesota "by all odds the commonest of the large tapeworms of man" seems pretty thoroughly substantiated.

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ON ZOOPHILISM WITH ANOPHELES—A REVIEW

L. O. Howard

The early work of Grassi (altogether the best and most complete for many years) showed that these mosquitoes prefer the blood of mammals, although they sometimes suck the blood of birds. He did not point out any preference for special mammals, but thought that the larger animals attracted them most. He laid this to the olfactory sense of the mosquitoes, and showed as illustrative of this that when a man and a horse were standing near each other the horse would be bitten many times before the man would be bitten at all; and again, when a man and a rabbit were near each other the man would be bitten before the rabbit. The preference of Anopheles for stables was frequently observed by some of the earlier writers like Kinoshita in Formosa and Mühlens in Northern Germany.

In 1920, E. Roubaud published an important paper on the conditions of nutrition of Anopheles in France and the part that domestic animals play in the prophylaxis of malaria. He had done earlier work on the infective power of the Anophelines that occur in France, and had noticed the existence of Anopheles without malaria and a diminution of malaria in many of the previously infested regions of western Europe, and had indicated that this condition of affairs was not due to any acquiring of immunity by *Anopheles maculipennis*. While recrudescences of malaria occurred in different parts of Europe on the return of malarious troops from abroad after the war, these recrudescences in France were insignificant. Roubaud judged that *Anopheles maculipennis,* although wide-spread and abundant in France, is not very pathogenic in that country, owing possibly to some peculiarity in its life history; in fact, the statement has been made, concerning Anopheles in England and in France, that they seldom or never bite human beings. The species under consideration, while almost a household pest in Algeria and in the Vendée in France, seldom bites man in the neighborhood of Paris. The temperature, of course, has something to do with this.

But Roubaud, in his 1920 paper, concludes that the key to the problem is the relative value of man and animals in the nutrition of Anopheles. Making intensive studies in the Vendée, he concluded that the Anopheles inhabit buildings of human construction occupied by either man or animals and that they seek these buildings not only for rest but for nourishment; they bite most frequently inside buildings; observation showed that the attraction to the Anopheles of the part of

a dwelling occupied by human beings is insignificant as compared with the great numbers that are found in the portion of a house where there are domestic animals. Thus human beings play an unimportant part in the nutrition of mosquitoes; cattle, horses and mules being favorite hosts; pigs, goats and sheep less attractive; rabbits of less importance, and dogs and fowls still less. With *Anopheles maculipennis,* he concludes that the maintenance of cattle and horses close to human dwellings gives man almost complete protection. Examinations of the blood in the digestive tract of mosquitoes showed that 14.2 per cent. had fed on human beings on the average, while frequently more than 90 per cent. contained the blood of domestic animals. In the summer, when the animals are in the open field, men are attacked; but even then the mosquitoes seem somewhat loath to bite the human species and apparently do not satisfy their hunger. Mentioning the point that certain authors think that animals are an inimical factor as encouraging the rapid multiplication of mosquitoes, he decides that this is not true, but that multiplication is almost entirely due to the presence or absence of favorable breeding grounds. He thinks that the Anopheles have been substituting animals for man as hosts and that this has been a slowly acquired habit, and that this adaptation of *Anopheles maculipennis* to animals in countries where domestic cattle abound has produced a race of this species of mosquito which is distinguished not only by this food habit but also by greater size. This change of habit, he thinks, in regions where cattle abound, has broken the cycle of the malarial parasites and suspended the endemic disease. He therefore thinks that the introduction of animal hosts and the study of the necessary conditions of stabling in accordance with the habits of the species of Anopheles are antimalarial measures. Showing that *A. maculipennis* seeks its hosts in rooms or closed buildings, *A. bifurcatus* bites in open air or under sheds, while other species attack equally well in or out of doors, he thinks that when the local modifications are understood this new measure will help considerably toward the elimination of malaria.

This paper of Roubaud's was considered by Wesenburg-Lund in 1921. This writer in Denmark has never found *Anopheles maculipennis* in a wild state. He had always found it in pigsties, cow-sheds and stables, which it never leaves except to mate and lay eggs. It can hardly be induced to bite man. He points out the marked contrast between this condition and the vigor of the same species in Italy and its voracity for human blood. However, a hundred years ago distressing epidemics of malaria occurred in Denmark, but during this hundred years domestic animals in that country have become consistent house dwellers. He thinks that there has arisen in Denmark a race of

Anopheles maculipennis which has a marked preference for the blood of animals. He also thinks that there has been an increase in size, since he has compared the measurements of the modern individuals with those in the descriptions of 120 years ago.

Later the same author amplifies this theory. He thinks that in Europe north of the Alps, on account of the abundant nourishment in cattle sheds, the species has increased in size and changed its habits; while south of the Alps, where the domestic animals spend most of the year out of doors, malaria is still rife and the species has retained its habit of biting man. He thinks that this change has occurred with great rapidity, and thinks that the *A. maculipennis* which live north of the Alps are not a distinct subspecies or local race but simply a contingent of individuals which have developed different physiological features. Osterwald and Taenzer, in September, 1920, state that in Germany Anopheles occurs chiefly in cattle sheds, preferring warm, dry sheds.

M. Sella, in 1921, reported concerning an antimosquito campaign in a village in the province of Caceres, Spain. Anopheles crowded into the outlying stables of the village, and practically none reached the houses during the spring. In June, however, they became numerous in the houses, but this increase did not entirely coincide with the removal of the animals. The author thinks that the invasion of houses by Anopheles is not only influenced by the number of domestic animals in the neighborhood, but also by the season and the resulting changes in the atmosphere of the houses and stables; the conditions that make them prefer stables to houses in winter persist throughout an appreciable part of the spring.

The brothers Sergent, writing on malaria in Algeria, in November, 1921, state that it is always stables and shelters for animals in Algeria that are first examined for Anopheles, and that they are always found there in greatest numbers. It seems that in Algeria animals are always kept in close proximity to houses as a precaution against thieves, but the Anopheles is so numerous that the animals do not protect the houses from malarial mosquitoes. Similar observations have been made by Baubault in Savoy, and by Eugling in Albania. There, the latter says, low goat sheds are preferred by Anopheles as shelters. Sheds built of wood and straw harbor thousands of mosquitoes, where those constructed of stone only, contain a few. Down in Sumatra, Schuffner and Hylkema, studying malaria during construction of an ocean harbor, point out the value of buffalo sheds to trap malarial mosquitoes. They found that one buffalo may divert most of the *Anopheles ludlowi* from the dwellings of one hundred people.

Martini, of Hamburg, published a paper in October admitting the value of cattle in protecting man from Anopheles, but denying that Anopheles has changed its habits in north Europe. Legendre, in October, 1921, reported that *Anopheles maculipennis* prefers the blood of rabbits, and advocates the keeping of rabbits as a means of protecting man.

Late in 1921, Roubaud, in answering objections that had been raised to his theory, attempted to show that natural selection has worked in a morphological as well as a physiological way with *A. maculipennis* and particularly with the teeth on the saw-like edge of the maxilla. In malarial regions the number of teeth averages about thirteen, whereas in the more northern regions, where the mosquitoes feed exclusively on cattle, an average number of fifteen teeth is found; and again, in other regions as many as eighteen teeth are found. This occurs in regions where the mosquitoes are especially abundant and it is more difficult to find cattle. He suggests that an examination of the number of teeth will indicate the liability to malaria in a given district. Where the teeth are few, the region is malarious; where they are numerous, malaria is scare or lacking. On the basis of this morphological change, he disagrees with Wesenburg-Lund in his conclusion that the change of habit was rapid.

In January, 1922, the Sergent brothers, Parrot and Foley published a note indicating that observations in Algeria do not support Roubaud's theory that the number of teeth on the maxillae has a relation to the intensity of malaria, indicating counts of over a thousand females of the species in Algeria which showed a variation of three in the number of teeth on the two maxillae. M. Langeron at the same time published a note in which he considered that Roubaud's theory was not definitely established. He advanced the idea that the reason that mosquitoes are found in large numbers in stables is because these places are dark and moist whereas human habitations are likely to be dry and well ventilated. He thought that the morphological change in regard to the teeth on the maxillae was hardly possible in so short a period. In his opinion, the disappearance of malaria has been due to the draining of deep ponds, the building of better roads, the better sanitary conditions of houses, and better food for the people. He did not deny the protective rôle played by cattle, but thought that this rôle is very limited, and advocated in the malarial parts of France the amelioration of living conditions of the people and the administration of quinine. Replying, Roubaud points out that his own deductions were not to be considered as final; that they only indicated certain conclusions. He thought that the actual variation as pointed out by the Algerian workers was after all slight, and that his theory is not verified in Algeria and can be accepted only

after an accurate comparison of further observations in different regions and after a uniform method of counting teeth has been adopted.

Later in the year Martini of Hamburg pointed out that after all there appears to have been no marked change in habit. According to the observations of the older writers on Diptera, *Anopheles maculipennis* seems to have behaved formerly just as it does now. He stated that the fact that biting is not observed proves nothing, as the attack occurs at night and is painless. In fact, he shows that one of the chief difficulties of those engaged in combating malaria in the Balkans was that the troops were not inconvenienced by the mosquitoes. He thinks that there is no basis for the hypothesis that a new race has been developed. He shows that individuals developed at a low temperature are often larger than those developed at a high temperature, and that probably the larger individuals will have on the average a larger number of teeth. He thinks that at present there is no justification for ascribing such variations to racial differences. He believes that the value of cattle in protecting man is an established fact, and states that he showed in 1916 that in one case malaria and other patients remained in the same barracks without indoor infection, because the mosquitoes were so strongly attracted to the military stables across the road.

Later in the same year Grassi published statements to the effect that *Anopheles maculipennis* varies in its tastes in different localities, and variation in food may be one of the factors that influence the epidemiology of malaria. He does not believe in the theory of protection against malaria through the presence of cattle, and shows that in portions of Italy where malaria is prevalent the number of cattle is no less than in other parts of the country where the disease is quite unknown. He compares two localities, in the one of which salts are more abundant in the waters than in the other, and thinks that the virulence of Anopheles is aggravated by the salinity of the water in which they breed. In the present year, F. W. Cragg, writing in India, believes in the protective influence of cattle. He points out that epidemic malaria in India has been noticed to follow heavy rainfall immediately after periods of deficient rainfall and famine; in the two famines of the last century more than half of the cattle were lost, and Cragg suggests that this loss of protective cattle must have been an important factor in the epidemic malaria which has followed famines.

After the publication of the articles just cited, the question of the actual feeding of *Anopheles quadrimaculatus* was carefully analyzed following collections and observations made by Dr. W. V. King at Mound, La. By the use of the precipitin method, a large series of tests was made by King and C. G. Bull with freshly fed mosquitoes collected under natural conditions during the summer months of 1922.

The blood contained in the midguts was tested against anti-sera for man and certain of the common domestic animals. Of the specimens found resting inside the houses, the majority had fed on human blood, and of those resting underneath the houses and in out-buildings the majority had fed on horses, cattle and pigs, with smaller numbers on dogs, chickens and cats. The lowest feeding rate on man occurred in the houses having the largest number of Anopheles, and the highest rate in those with the lowest density of Anopheles. Three plantations were studied, the houses being for the most part negro cabins. The results varied from 2 to 7.2 per cent on humans, from 27 to 40 per cent. on horses, 24 to 47 per cent. on cattle, 13 to 21 per cent on pigs, with about 11 per cent. on other animals.

During August and September, C. G. Bull and F. M. Root conducted some interesting tests. In each test several hundred bred female mosquitoes were liberated about 6 p. m. in a mosquito-proof shed divided into six stalls in which men and domestic animals were exposed to the bites. The next morning the engorged mosquitoes were collected, taken to the laboratory, and the blood in their stomachs was identified by the precipitin test. In every experiment in which men (in bed, with only head and arms exposed) were tested in competition with horses or cows, some mosquitoes fed on the men although two or three times as many always fed on the domestic animals. These experiments were inconclusive, on account of the much greater exposed surface presented by the animals. With horses and cows alone, there were indications that a certain cow was twice as attractive as a horse, while another cow was only half as attractive as the same horse. All this indicates the great desirability of additional experiments on a much larger scale.

The whole question has aroused so much interest in various parts of the world, and especially in Europe, that it becomes important that further studies be made in the United States. The investigation of differential feeding habits will undoubtedly be carried further by Doctor King and his Johns Hopkins colleagues; and the maxillary dentation question and its morphological, taxonomic, and evolutionary significance will be taken up by Doctor Dyar and Mr. Shannon. The latter, at my suggestion, has already begun preliminary work in this direction. He tells me that he has found in nine specimens of *Anopheles maculipennis* (*occidentalis*) from a single California locality a variation in the number of teeth from thirteen to eighteen; that three specimens of *A. punctipennis* from Plummer's Island show fifteen teeth each; and twelve specimens of *A. albimanus* from Panama show a range of from thirteen to seventeen. This work will be continued on a very much larger scale and over a wide distribution, with the possible differences carefully noted between malarious and non-malarious regions.

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ADDENDUM

While this manuscript was in the hands of the printer there appeared an important article entitled "Notes on Relation of Domestic Animals to Anopheles," by M. A. Barber and T. B. Hayne of the U. S. Public Health Service, published in Public Health Reports, Vol. 39, No. 4, January 25, 1924, pages 139-144. The authors recite certain experiments that they have carried on, and show that in the United States *Anopheles quadrimaculatus* and *A. crucians* appear to "show no special predilection for domestic animals over man when such factors as size and amount of exposure are included." The conditions described by Roubaud as obtaining in France are not present in this country—at all events, not in the southern states. "It is questionable whether the increase in animal industry, apart from drainage and other concomitant improvements, has been a large factor in the decrease of malaria which has occurred in many parts of the United States."—L. O. H.

THE REACTIONS OF THE MIRACIDIA OF *SCHISTOSOMA JAPONICUM* AND *S. HAEMATOBIUM* IN THE PRESENCE OF THEIR INTERMEDIATE HOSTS *

Ernest Carroll Faust

A study of the several phenomena connected with the life-histories of the human blood-flukes is no less interesting from the biological standpoint than it is important in the elucidation of the practical aspects of human infection with these flukes. Among these phenomena the relation of the miracidium to its free-living environment and its reaction to the molluscs that serve as its intermediate hosts are the particular problems involved in this paper. I have had the opportunity of studying living miracidia of the Oriental blood-fluke, *Schistosoma japonicum*, both from Chinese and Japanese sources. I have observed the various stages of development from the immature larva within the egg, through the hatching phenomena and the free-living period, to the attack on the susceptible mollusc submitted to infection, and finally culminating in the penetration of the mollusc. Certain of the observations were so unique that it seemed advisable to make similar observations on the miracidia of the Egyptian blood-fluke, *Schistosoma haematobium*. The latter study was made in Cario, Egypt, where facilities were afforded through the courtesy of Doctor Mohammed Khalil, helminthologist in the Public Health Department.

The eggs of *Schistosoma japonicum* as deposited by the female worm in the radicles of the mesenteric veins are immature (Fig. 1) but by the time they are evacuated in the feces they are usually far along in development (Fig. 2) and on dilution of the stool with tap water, hatch in from 2 to 24 hours (Fig. 3). After swimming around in the bottom of the container for a brief period they rise to within a few millimeters of the surface, where they move about rapidly for twenty-four to thirty hours. In the event that the specific mollusc host is not present in the immediate vicinity during this infective period, the miracidia, having used up all their stored energy, sink to the bottom of the container and die. On the other hand, if the susceptible mollusc is present a reaction on the part of the larvae is observed as soon as they come within a few millimeters of the snail. Careful study has revealed

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the fact that in order to cause this reaction the mollusc must pass through the subsurface stratum in which the miracidia are congregated. It seems probable, therefore, that snails which usually live some distance under the surface of the water would not ordinarily be subject to infection, even if they were capable of stimulating an attack by the miracidium.

Once a miracidium of *Schistosoma japonicum* has come within stimulating range of the proper snail it makes a bee-line drive for that snail and attacks it at the first place of contact. The detailed data on this phase of the problem are the subject of another paper (Faust and Meleney, 1924). If the shell is attacked, penetration is, of course, impossible. But all muscular parts of head and foot, which usually protrude from the shell, as well as the organs within the mantle cavity, can be readily penetrated. One-half hour after a successful attack has been launched, miracidia may be found to have penetrated several times their body distance into the tissues of the head or foot. The mechanism of penetration of the mollusc is primarily that of a digestion of the host tissues by powerful secretions, which are probably proteolytic ferments, poured out by paired glands giving respectively basophilic and oxyphilic reactions. An hour after penetration has been effected necrotic areas can be demonstrated in the host tissue immediately around the miracidia. Thus an artificial lymph space in the loose mesenchymatous tissues of the host is secured which sooner or later opens into a natural lymph sinus. While the ciliated ectoderm covering the miracidium of *S. japonicum* is probably utilized only during the free-living stage of the larva, it is not shed previous to penetration of the snail. The corresponding phenomena of hatching, free-living existence, attack and penetration of the susceptible snail on the part of the miracidium of *Schistosoma haematobium* belongs to the same general type described for this period in the life-history of *S. japonicum*. However, these successive events all differ specifically just as much as the detailed morphological features of eggs and miracidia serve to differentiate these two species of worms.

The eggs of *Schistosoma haematobium* are deposited by the female worms in the vesical plexuses of the portal veins, at which time they contain immature larvae (Fig. 4). By the time they have filtered through the muscular and glandular tissues of the bladder they have developed to maturity (Fig. 5). The eggs are voided in the urine and usually contain vigorous miracidia. On centrifuging the urine and diluting with distilled water hatching is effected almost immediately (Fig. 6). The miracidia swim about for a time in the bottom stratum of the container, but after a little while they distribute themselves throughout the container. In order to determine if there was any par-

ticular stratum of water in which they particularly congregated, viable eggs from the urine of an infected patient were centrifuged slowly for two minutes and then washed several times in lukewarm tapwater. They were then placed in cylinder glasses and allowed to hatch. Observations were made from time to time on the number of miracidia collecting at various levels of the water. Several counts from several samples of material demonstrated that there was an equal distribution of the miracidia throughout the medium. Specimens of a susceptible molluscan host were then placed in the bottom of the containers and the activities of the miracidia studied. *The snails were attacked* almost immediately *at all levels of the water column,* and two hours later the miracidia apparently had all penetrated the snails for they had disappeared from the water.

These observations indicate that the behavior of the two species of miracidia differs in this respect, that the larvae of *Schistosoma japonicum* collect very soon after hatching near the top of the water column and are in a position to infect only these snails found in this stratum. On the other hand, the larvae of *S. haematobium* are quite equally distributed throughout the water and are in a position to attack at any level the snails to which they are adjusted.

Penetration of the snail on the part of the miracidium of *Schistosoma haematobium* is very similar to that of the miracidium of *S. japonicum.* Immediately a group of snails of the species *Isidora* (*Bullinus*) *contorta* or related species is placed in a medium in which the miracidia are swarming, the snails are vigorously attacked at the first point of contact and penetration attempted. If this point, is soft body tissue, penetration is usually successful. Sections of *Isidora contorta* which I submitted to infection for one hour with miracidia of *S. haematobium* at Cairo in July showed penetration of the thickened portions of the mantle fold, head and foot. In all cases examined the larvae had reached the loose mesenchymatous tissues. During the attack the snails were characterized by the jerky nervous movements which have been described for this and other species of molluscs at the time of attack and invasion.

The operculate snails, *Katayama nosophora* (Fig. 8) was first discovered to be an intermediate host for *Schistosoma japonicum* by Miyairi in Japan (1913). Many investigators since that time have confirmed their data. The first discovery of an intermediate host in China was made last year by my colleague, Doctor Henry E. Meleney, who demonstrated that *Oncomelania hupensis* (Fig. 7) was the species involved in the vicinity of Soochow. More recently I have found this same snail to be involved in another endemic center, while in a nearby area of infection I have found *Katayama nosophora* to harbor the

worm. The mollusc known to serve as intermediate host in Formosa is *Katayama formosana* (Fig. 9). The particular group of operculate snails to which these forms belong is amphibious in habits and conforms to the specialized requirements of being present for the greater part of the time at or near the water level. If these snails are placed in the bottom of an aquarium they immediately crawl to the top. They prefer to live among the decaying vegetation on moist banks of quiet streams. The specialized adaptation of the miracidia of *S. japonicum* precludes them from using other groups of operculate or non-operculate snails as hosts, even if the stimulus to which the miracidia reacts were the same.

Isidora (*Bullinus*) *contorta* (*truncata*) (Fig. 10) was first shown by Leiper in 1915 (1918) to be involved in the life-history of *Schistosoma haematobium* in Egypt. *I. dybowski* and *I. innesi* are incidentally involved. *I. contorta* also harbors the infection in Mesopotamia, Palestine, Syria, Tunisia, Algeria, Morocco, France and Corsica, while in South Africa *Isidora* (*Physopsis*) *africana* (Fig. 11) a closely related species, is incriminated. The major responsibility for propagating the fluke in Portugal and Morocco rests with *Planorbis metidjensis* and *Planorbis dufourii*. All of these species belong to the family Planorbidae.

It has been shown (Faust and Meleney, 1924) that miracidia of *Schistosoma japonicum* which under ordinary conditions select *Katayama nosophora* for attack and penetration can utilize *Oncomelania hupensis* equally well. The reverse experiment has been entirely successful, namely, miracidia of *S. japonicum*, which under natural conditions select Oncomelania, can infect Katayama. While this phenomenon has not been demonstrated for the species, *S. haematobium*, it seem altogether probable that any miracidum of this worm can utilize any one of the susceptible hosts for attack and invasion. The relationship between parasites and host seems, therefore, to be a group phenomenon. Sewell's supposition (1919) that a cercaria found by him in Calcutta was probably the larval stage of *S. japonicum* can be discarded entirely on grounds of host relationship, since it was found in a planorbid snail, which belongs to an entirely different host group than that which *S. japonicum* utilizes. The reaction of the miracidia to the molluscan host is believed to be a chemotactic one. This chemotactic stimulus probably lies in the mucus secreted by the snail. It is the mucus-secreting parts of the snail which are most usually attacked. Furthermore, I have observed miracidia of *Schistosoma japonicum* attacking a trail of mucus secreted by Katayama as vigorously as they did the mollusc itself. If this is the case, then the entire problem of interrelation between miracidum and molluscan host is one of specificity of mucus secretion.

After an overwhelming invasion of a susceptible molluscan host by human blood-fluke miracidia there is at least a short period of time when no further infection is possible. When molluscs which have been infected only a few hours previously are placed in a container, in which miracidia of the same species are swimming about they are not attacked by the new group. Some days later, however, a second invasion may occur. Whether this phenomenon is similar to that of the fertilized ovum and the sperm or is based on some other biological reaction remains to be determined.

Altogether the relationship of the miracidia of the human blood-flukes to their specific molluscan hosts is a very precise one, and requires an exact adjustment of the two organisms involved as well as an environmental balance. On the other hand wide range of accommodation which these larvae have for temperature and H-ion concentration stands out in direct contrast to the host-parasite mechanism. Although the miracidium develops at 37° C. within the mammalian body and although growth is retarded at a temperature slightly lower, when once mature, hatching may take place in a medium as cold as 4° C. Moreover, the molluscan host is a cold-blooded animal and the intra-molluscan phase of the life-history proceeds normally at this lower temperature. Again, the host, Katayama, lives in water with a P_H near 6.78, while Oncomelania normally develops in water with a P_H approximating 7.20. Yet the miracidum from a strain of blood-flukes accustomed to the Katayama medium can readily adjust itself to the Oncomelania environment. However, this is less remarkable than the change of the miracidium from a free-living existence to the H-ion concentration of the serum which bathes it in the lymph spaces of the molluscan body ($P_H = 7.60$).

SUMMARY

1. A comparative study of the reactions of the miracidia of *Schistosoma japonicum* and *S. haematobium* in the presence of their intermediate hosts has thrown light on the exact relationships obtaining in these respective host-parasite cycles.

2. The free-swimming infective miracidia of *Schistosoma japonicum* move about in the top stratum of water. Only these molluscs which pass through this stratum are exposed to infection, even if they were otherwise capable of stimulating an attack by the miracidia.

3. The free-swimming infective miracidia of *Schistosoma haematobium* are equally distributed throughout the water in which they hatch. All snails in the immediate vicinity capable of stimulating an attack by the miracidia are likely to be attacked and infected.

4. Several closely related species of operculate snails belonging to the genera Katayama and Oncomelania have been found to harbor *Schistosoma japonicum* infection.

5. Several closely related species of non-operculative snails belonging to the genera Isidora and Planorbis have been found to harbor *Schistosoma haematobium* infection.

6. The active mechanism of the miracidium for the specific molluscan host is probably a chemotactic one, in which the mucus secretion is the substance involved.

7. Although the host-parasite mechanism is based on a precise adjustment, the miracidium is capable of a very wide accommodation as regards temperature and H-ion concentration.

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Description of Plate XXII

Fig. 1.—Immature egg of *Schistosoma japonicum*. × 566.

Fig. 2.—Mature egg of *S. japonicum*. × 566.

Fig. 3.—Hatched miracidium of *S. japonicum*. × 566.

Fig. 4.—Immature egg of *S. haematobium*. × 210. After Bettencourt and Borges.

Fig. 5.—Mature egg of *S. haematobium*. × 260. After Bettencourt and Borges.

Fig. 6.—Hatched miracidium of *S. haematobium*. × 500.

Fig. 7.—*Oncomelania hupensis*. × 5.

Fig. 8.—*Katayama nosophora*. × 5.

Fig 9.—*Katayama formosana*. × 4.

Fig. 10.—*Isidora contorta*. × 2.

Fig. 11.—*Isidora africana*. × 2.

PLATE XXII

CAPILLARIA COLUMBAE (RUD.) FROM THE CHICKEN AND TURKEY

H. W. Graybill

During a period of several years in making autopsies on chickens and turkeys, the writer has found on a number of occasions a species of trichosome in the small intestine. This worm has been encountered only in small numbers and in birds showing no macroscopic changes of the intestine. It is known however that species in this genus attach themselves to and even penetrate the mucosa of the alimentary tract and that when present in large numbers they may be responsible for serious disease and death, especially in young birds. Freese (1908), Reibisch (1893) and Perroncito and Tomiolo (1899), cited by Neveu-Lemaire (1912), as well as other authors have reported disease in birds due to trichosomes, the former author in chickens due to *Trichosoma retusum*, and the latter authors in pheasants due to *Trichosoma strumosum*.

A number of species of trichosomes have been reported from the chicken. Neveu-Lemaire refers to the following: *T. retusum* Railliet, 1893; *T. collare* von Linstow, 1873; *T. caudinflatum* (Molin, 1858) Stoss., 1890; *T. annulatum* Molin, 1858; *T. dubium* Kowal., 1894, and *T. gallinum* Kowal., 1894. Stubbs and Crawley (1922) report a *Capillaria* sp. from the intestine of the chicken and attribute a chronic proliferative enteritis to it. Travassos (1915) reports *Capillaria strumosa* (Reibisch, 1893), a parasite of pheasants, in the chicken. Barile (1912) reports from the intestine of the domestic turkey, under the name of *Trichosomum meleagris-gallopava*, a species showing certain points of relationship to *T. retusum*.

As will be shown presently, the form collected by the writer proved not to be one previously reported from the chicken or turkey, but *Trichosoma columbae* Rud., 1819, parasitic in the large and small intestines of the domestic pigeon and certain other columbines. The meager accounts of this species warrant the publication of the more complete study which the writer has made.

DESCRIPTION OF THE PARASITE

The worms have a whitish, translucent appearance, and because of the great thinness of the body it is very difficult to see them in the mucus when the intestine is opened. As a rule it is only when mounts of the intestinal contents are made or the contents have been subjected to washing and sedimentation that they are discovered. The cuticula is

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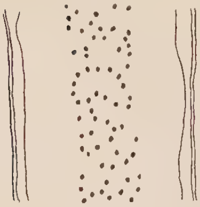
finely striated transversely. The anterior end is thin, bluntly pointed, and nude. There are fairly broad lateral bacillary bands (Fig. 1) which become narrower and less distinct toward the extremities. The cells of the esophagus are rectangular in outline, annulated, and have a prominent nucleus located at the middle.

The males are less numerous than the females. Two measured 8.6 and 10 mm. long. The esophagus is about one-half the length of the body. The maximum width (53μ) occurs approximately at the junction of the middle and posterior thirds of the body. The body tapers in both directions, anteriorly to a comparatively fine filament, but only slightly toward the posterior end. The cloacal aperture is nearly terminal. It is surrounded dorsally and on the sides by a small, scoop-shaped, transparent bursa which is a continuation of the body (Figs. 2, 3). It is not lobed (Fig. 4) and is supported on each side by a broad, rounded ray which is constricted somewhat at its base. The sheath of the spicule in the smaller of the two worms measured was 2.5 mm. long. It was uniform in width (12.3μ) and marked with transverse striae (Fig. 5). The striation may be indistinct or wanting in places. The measurements of the spicule in the above specimen was 1.2 mm. long and 8.2μ broad. It is rounded at the distal end and expanded like a trumpet at the proximal end (Fig. 6).

In the female the anterior half of the body is whitish and translucent, the posterior more or less dark and opaque due to the presence of the genital organs and ova. Three measured 10, 10.7 and 12.3 mm. long. The maximum width (79μ) occurs toward the posterior end. Anteriorly the body tapers as in the male, posteriorly it tapers a little and the end is broad and round. In the first two of the above specimens the esophagus measured respectively 4.6 and 5.6 mm. long. In both sexes the esophagus near the anterior end suffers a sudden reduction of about one-half in width and then tapers to a very fine tube anteriorly. This region of the esophagus was 328μ long in a male and 352μ in a female. The anus is slightly subterminal. The vulva is situated near the junction of the esophagus an intestine. The vagina passes backward (Fig. 7).

The eggs (Fig. 7) are slightly brownish in color and ellipsoidal in shape. The outer layer of the shell is highly refractive and 1.7μ thick. It is discontinuous at the poles forming there a neck-like structure about a circular aperture. It is uniform in thickness except at the poles where it is somewhat amplified. The openings at the poles are closed by transparent dome-shaped plugs. The inner layer of the shell is only a fraction (one-third) of the thickness of the outer. It is in contact with the latter except at the poles where it disengages itself forming a complete ellipsoidal envelope, although it may be indented at one or both poles. The ovum is granular in structure and



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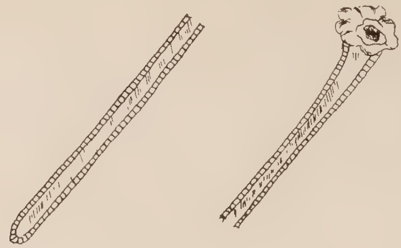

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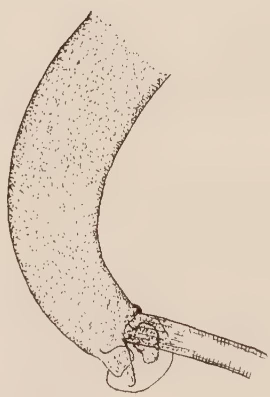

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EXPLANATION OF PLATE XXIII

Drawings made with a camera lucida

Fig. 1.—Surface view of body of female at posterior end of esophagus showing lateral bacillary band. × 80.

Fig. 2.—Posterior end of male showing bursa, its supporting rays and the proximal portion of sheath of spicule. × 345.

Fig. 3.—Another view of posterior end of a male. × 345.

Fig. 4.—Ventral view of the posterior end of a male showing the bursa, its rays and the proximal portion of sheath of spicule. × 680.

Fig. 5.—Distal end of sheath of spicule. × 345.

Fig. 6.—Proximal and distal portions of spicule. × 345.

Fig. 7.—Lateral view of body of female in region of vulva showing posterior end of esophagus, vagina and uterus containing ova. × 345.

nearly fills the space within the shell. It is deposited in an unsegmented condition. The eggs measure 50 to 62μ long and 20 to 27μ broad.

Gravid females were cut up in a shallow layer of physiological salt solution in small Petri dishes to liberate the ova. The cultures were held at room temperature. In a culture held at a temperature of 22 to 25° C., the ova developed coiled vermiform embryos in 7 days. Another culture held at a temperature of 24 to 25° C. developed in 6 days.

In seeking to determine whether the parasite studied might be one described from birds other than chickens and turkeys, the writer was impressed with its similarity to the description of *Trichosoma columbae* Rud. 1819, as given by Railliet (1893), and of *C. dujardini* Travassos, which evidently is the same species renamed. Material collected from domestic pigeons in Washington * and also near Princeton was examined and found to correspond with the descriptions referred to, so it seems safe to consider the American form *C. columbae*. A comparison of the form described with that of the pigeon showed their identity.

Travassos (1915) has failed to describe the bursa. He gives the length of the spicule as 1.5 to 1.57 mm. and the width as 10μ. The writer has measured the spicules in eight males collected from pigeons in this country and has found them to range from 1.3 to 1.58 mm.

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* This material was furnished through the kindness of Dr. B. H. Ransom, of the Zoological Division, U. S. Bureau of Animal Industry.

SOCIETY PROCEEDINGS

HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The sixty-sixth meeting of the society was held on Feb. 17, 1923.

Messrs. Cobb, Steiner and Christie presented an extended paper on *Agamermis decaudata n. sp.* (since published, Jour. Agric. Research, 28:921-926).

In discussion, Dr. Stiles raised the question of attack of beneficial insects by mermithids. He also noted that culicids in certain localities are more or less controlled by a mermithid. Dr. Steiner pointed out that mermithids live in low, wet places where beneficial insects are few. He also stated that simulids in the High Alps are controlled by a Mermis. Dr. Bartsch stated that two injurious snails have been introduced from Bermuda into Texas, where they are attacking the Easter lily and onion and that as yet no control measure had been found. Possibly they could be controlled by mermithids.

Dr. Chapin reported *Typhlocoelum flavum* from the Canvas back and Red head ducks from Currituck Sound, N. C. Also the presence in the United States of a schistosome fluke of the genus *Ornithobilharzia* in the mesenteric veins of *Marila affinis,* from Shadyside, Md., was noted.

The sixty-seventh meeting of the society was held on March 17, 1923.

Dr. Hall presented the following note by Dr. A. C. Chandler:

A Note on *Ascaris lumbricoides with Three Uteri and Ovaries*

The writer's attention was called recently to an abnormality in a specimen of *Ascaris lumbricoides,* of pig origin, which is of more than usual interest from an evolutionary standpoint. The specimen was being dissected by a freshman student who was very much disturbed to find that his Ascaris had three instead of the orthodox two uteri. Unfortunately the vagina had already been torn free from the vulva, but otherwise the specimen was in good condition, and has been sent to the Helminthological Collections of the U. S. National Museum (B.A.I. No. 25232).

On closer examination it was found that this specimen actually had three complete uteri and three ovaries, all about equally developed, and fully as long as the organs of a normal individual. At the end of the vagina there is a bifurcation into a single normal uterus and a double branch, the bifurcation of the latter extending almost, but not quite, to its junction with the vagina, as shown in an accompanying sketch.

The existence of this unusual abnormality in a specimen of *Ascaris lumbricoides* seems to point to a rather deep-seated tendency on the part of this group of nematodes to a multiplication of the uterine branches, a tendency which finds permanent expression in the development of species in reptiles having four- or six-branched uteri (genus Polydelphis).

Dr. Hall also presented a note by Dr. Chandler on speciation and host relationships of parasites (since published in Parasitology, 15:326-339). In discussion of this paper Dr. Cort noted that he had examined flukes assigned to *Clonorchis sinensis* and *C. endemicus* and finds, like Kobayashi, that a difference in size is the only constant difference; he thinks a trinomial name might be applied in this case. He also noted that the varietal names *major* and *minor* have been used, but thinks, Dr. Stiles concurring in this opinion, that these names are not in order. He finds specimens from the cat to be only half as large as the human fluke. Dr. Stiles expressed some doubt as to whether size alone is sufficient ground for even varietal recognition. Dr. Cobb emphasized in this connection the necessity for accuracy of observation and description.

Dr. Hall presented the following notes:

Intrauterine Infestation of Dogs with Hookworms

Dr. J. Wm. Fink of Newburgh, N. Y., reported under date of October 6, 1922, that in dealing with a hookworm infestation in pups he found hookworm eggs in the feces of pups 1 to 2 weeks old and mature hookworms in the intestine postmortem in pups 3 weeks old. As hookworm eggs appear in the feces in 4 to 5 weeks in experimental infestations, these findings point definitely to prenatal infection. Adler and Clark (1922, Ann. Trp. Med. Hyg., 16:353-354) report natural hookworm infestations in six pups, 5, 13, 14 and 15 days old, with worm eggs present in the feces from the three older groups (four animals).

Heterakis dispar in the United States

Among some parasites sent in by Dr. Van Es from Lincoln, Nebraska, there were some specimens of *Heterakis dispar* from the goose. A casual examination of the literature does not disclose any definite record of this parasite from this country. Verrill (1870) gives it in his list but with no indication of its occurrence. According to Lamon and Lee (1917) the 1910 census showed that there were 400,000 geese in Kentucky alone, with an almost equally large number in Tennessee, Missouri and Arkansas, indicating an industry of sufficient magnitude to warrant a study of the possible losses from parasites.

Multiple infestation with Cuterebra larvae in cats

In 1921 the writer reported cases of Cuterebra larvae in cats, summarizing the cases recorded up to that time. Over 9 such cases were reported. Three additional records were noted in 1922. One of the 1922 records was a case reported by Dr. M. J. Hughes of Boontown, N. J. Dr. Hughes wrote under date of August 24, 1922: "You might be interested to know that in a litter of kittens on the same premises there have appeared five more of the larvae, three under the skin of one kitten and two under that of another. I have removed them, treated the cavities antiseptically in each case and have had good cures." In one of the cases noted in 1921 as reported in Insect Life, larvae are reported as collected from the side of the eye and from the back of one cat. This case and the two from Boontown are the only cases of multiple infestation known to me, the largest number of larvae from one animal being three. This brings the total number of records of Cuterebra larvae from cats to over fourteen. Apparently dogs are less susceptible to this parasite than cats, there being but one record (French 1893) from the dog.

Dr. Hall presented the following note by Ransom and Hall:

The turkey as a host of *Cheilospirura hamulosa*

This gizzard worm of poultry has been reported heretofore only from the chicken and pheasant *Phasianus gallus* according to the nematode catalogue of Stiles and Hassall. This worm was first reported from the United States by Ransom in a note presented before the Helminthological Society in 1911 and published in Science in 1912. In the collections of the Bureau of Animal Industry, there are specimens from the turkey *Meleagris gallopavo*, collected on three occasions, as follows: No. 18224, collected by Dr. Black, Washington, D.C., December, 1915; identified by Hall; No. 18320, collected by Dr. Avery, Vienna, Va., January, 1916, determined by Ransom; No. 18509, collected by Dr. Avery, Bethesda, Md., July, 1916, determined by Ransom.

Dr. Cobb presented the following paper, accompanied by an exhibition of specimens:

Interesting new genus of Nemas inhabiting nests of tropical ants

In the nests of certain tropical ants (found in the thorns, petioles, stems, etc., of certain tropical plants) there are interesting new genera of syngonic nemas related to the genus Rhabditis. The nemas are found in the so-called

latrines of the ants and feed upon fungi growing in the latrines. One species of ant prepares for its larvae balls of food compounded of fungous elements together with the eggs and remains of the nemas—a sort of *nema salad.* The nemas have developed labial dorso-ventral mandibles for biting off the portions of the fungus used by them as food—the spores and certain parts of the mycelium. The nemas show a high degree of adaptation to their environment and seem beyond question to be an integral part of the biocene, our conception of which thus becomes even more complicated and wonderful than before. The adaptation is so close that it seems unlikely these particular nemas will be found anywhere except in conjunction with the ants. The nemas belong to several species—sometimes more than one species being associated with a single species of ant. It is not thought rash to predict that other similar species of nemas will yet be found associated in a similar way with other species of ants. The nemas were furnished by Doctors Wheeler and Bailey of the Bussey Institution, and by Mr. Zetek of the U. S. Bureau of Entomology.

In comment, Dr. Stiles asked Dr. Cobb's opinion as to the advisability of including the mermithids in the Trichosyringata. In Dr. Cobb's opinion, the mermithids were as yet inadequately studied and it would be difficult to place them at the present time. Dr. Steiner noted that mermithids have relationships with the Dorylaimidae. Apparently mermithids should be omitted from a classification primarily concerned with nematodes of vertebrates, for the time being.

Dr. Shillinger presented the following notes:

An attempt to produce intrauterine infestation in swine

Embryonated eggs of *Ascaris lumbricoides* from swine were fed to a year old sow in advanced pregnancy on February 8, 10, 12, 14 and 17. On February 23, the sow had six pigs. The animals were killed and press preparations of the lungs, liver, spleen and kidneys examined. No larval ascarids and no lesions attributable to ascarid larvae were observed.

Attachment of one parasite to another in the host

On March 17, 1923, while performing a post-mortem examination on a dog, two specimens of *Ancylostoma caninum* were found attached to *Taenia pisiformis* by the buccal capsule. They were attached to mature and gravid segments. While such findings are probably not uncommon they appear to have been reported but a few times in the literature, and the available previous records are summarized here.

Grassi and Parona in 1879 have reported a case in which two specimens of *Ancylostoma caninum* were found attached to a specimen of *Taenia crassicollis* in a cat. Adler, 1922, reports three cases of *Ancylostoma caninum* found attached to *Dipylidium caninum.*

Specimens of *Syngamus laryngeus* collected in the vicinity of Manila, 1899, furnished the United States Bureau of Animal Industry through the courtesy of Dr. William Boynton showed a very interesting condition which was reported by Hall in 1915. In one pair of these worms in which the male was attached to the female in the usual way, the male was also attached to the neck of the female by the buccal capsule. In another case the male of one couple was attached by means of the buccal capsule to a point opposite the vulva of a female belonging to another couple. In both of these cases the powerful suction had drawn the cuticula of the female deep into the capsule but not quite in contact with the teeth at the base. The cuticula was unbroken and protected the viscera of the attacked worm.

Dr. Albert Hassall states that the attachment of one trematode to another is not uncommon, and that Rudolphi has made one trematode species which subsequent examination of the type material has shown to be based on a specimen showing a protuberance which was evidently due to a previous attach-

ment of another fluke. The protuberance which formed a distinguished characteristic of Rudolphi's species represented part of the attached fluke which had been drawn into the sucker cavity.

Dr. Shillinger exhibited the specimens referred to in his report. The hookworms had detached when they were placed with the tapeworm in an incubator and had reattached when returned to room temperature.

Dr. Cobb, in comment, raised the question as to the best solution strengths in which to keep nemas alive, noting that he had found normal saline unsatisfactory for free-living nemas. Dr. Ransom stated that Kronecker's solution, a slightly alkaline saline solution, was satisfactory for some worms, but that some required stronger solutions, up to 1 per cent. for trichinae larvae, and others required a weaker solution. Dr. Cort stated that he found it necessary to use weaker solutions for some parasites, 0.4 per cent. being satisfactory for some of these, cercariae and frog trematodes requiring these weaker solutions.

In connection with Dr. Shillinger's paper, Dr. Cobb reported that he had an apparently normal specimen of free-living nema in which the spicules approached the cloacal aperture from opposite directions; nevertheless these proved to be the *ingested spicula af another* nema. The appearances were very deceptive. Dr. Ransom noted that in a species of Nematodirus studied by Dr. May there was an apparent abnormality of the spicules, but it was subsequently found that the spicules had been extruded, as a result of some accident, in such a way that the proximal ends everted and the distal ends stayed in the body, the spicule being in this way reversed.

Dr. Davis presented the following note:

Intracellular Stages of a Fish Flagellate (printed in J. Parasit., 9: 153)

Dr. Cobb noted in connection with this that he had found certain nemas in prepared fish foods and had since found the same nemas in association with ants. He had been informed that pupae and larvae of ants are used in the preparation of fish foods.

Dr. D. M. Malloy, who has had extensive experience in tropical field work, reported his experience in the use of 606 in the Philippines in 1910. He was detailed to work among the Igorrotes and found a case of framboesia which he offered to treat. The patient expressed a preference for his own witch doctor and refused treatment. Subsequently the man was arrested for vagrancy and as a prisoner was given treatment. With the beginning of recovery he was highly gratified with the treatment. He was allowed to go home on his promise to return, and returned two days later with his entire village of 60 persons for treatment. An additional supply of 606 was requested by telegraph and a runner sent to the coast, 90 miles away, for this. The runner was back the third day with the drug, and fifty-eight cases of framboesia were treated, all successfully. In return Dr. Malloy was invited to the village and a three day feast held in his honor. These natives were head hunters and still had numerous souvenirs of the head-hunting habit.

In comment Dr. Stiles related some instances showing the primitive condition of existing beliefs among persons in certain sections of the United States.

Dr. Carr reported an instance in which the human ascarid was used as bait for tarpon with very satisfactory results. In comment, Dr. Hall stated that swine ascarids were obtained by negroes at slaughter houses in parts of the United States for use as bait to catch carp. Negroes say that these make very good bait for this purpose.

EDWARD A. CHAPIN, *Secretary.*

The sixty-eighth meeting was held on April 12, 1923. It was the annual dinner, with Professor Henry B. Ward of Urbana, Ill., as guest of honor.

The sixty-ninth meeting was held on May 19, 1923. Officers for the ensuing year were elected as follows: president, Dr. Chapin; recording secretary, Dr. Stiles; treasurer and recording secretary, Miss Cram.

Dr. G. Steiner exhibited specimens of *Geotrupes* sp. and another dung beetle both from Falls Church, Va., with cysts of Rhabditis, apparently *Rhabditis coarctata* Leuckart, located on the mouth parts and legs. The cysts are white, of cylindrical shape, with a long well set-off tail, and are fixed on the insect by means of a short peduncule. The Rhabditis larva forms a small, oval body inside, folded and shrunken. This is the first time such cysts have been observed in the United States. Specimens of *Aphodius fimetarius* and two dipterous larvae from cow dung forwarded by Prof. Dr. Müller of the University of Greifswald were also exhibited. Moniez first observed such cysts on Acarina, living also in cow dung from localities near Lille, France, and three years later they were described by Leuckart from Leipzig. Later Moniez again mentioned them from Geotrupes, Staphilinus, Thysanura and Myriapoda, which also live in cow dung. The cysts are a means of passive transportation and distribution through the help of the insect. They often are present in large numbers, especially on the mouth parts and legs of the insects, but in the case of the two mentioned insect larvae they covered also the segments of the body. Since these larvae may moult before leaving the cow dung, it seems that in this case the instinct mislead the nema larvae to an impotent carrier.

Dr. Chapin presented the following:

Note on *Spironoura affine* Leidy, 1856. In 1856, Joseph Leidy gave a very short description of a nematode that had been found in the cecum of the box tortoise (*Terrepene carolina*). While the description is not all that could be desired from present-day view-point, a comparison of it with material collected recently from that host and habitat near Washington, D. C., leaves no doubt that the worm described by Leidy is the common colon parasite of the box tortoise in this locality. While not the type of the genus Spironoura, it was one of the two originally included species and satisfies the requirements of that genus in every respect. *Falcaustra chapini* Boulenger 1923, is based upon material belonging to the same species; and if Boulenger is correct in his generic assignment, Falcaustra Lane, 1915, will probably fall as a synonym of Spironoura Leidy, 1856.

Dr. Stiles gave a summary of ground-water pollution experiments at Fort Caswell, N. C., showing that *Bacillus coli* had been traced for various distances up to 65 feet from the pollution trench, and uranin up to 115 feet. He correlated the extension of the bacteria with the rise and fall of the ground water table and this with the rainfall. A summary of the experiments has appeared in Public Health Reports, 38: 1350-1353.

Mr. J. Sandground presented a paper dealing with the probable identity of the eggs of *Heterodera radicicola* with those of *Oxyuris incognita*, which Kofoid and White have described from human stools. (This Journal, 10: 92.)

Dr. Cobb remarked that the geographic distribution of this species is in harmony with that of *Heterodera radicicola.*

Dr. Payne gave an informal discussion of certain investigations on carbon tetrachloride, requesting that the data be not recorded. In the discussion, Dr. Hall mentioned the effect of the drug on different species of hosts, swine not bearing the drug so well as do other animals; he mentioned enteritis as a contraindication; he referred to castor oil as a safeguard, but olive oil as a distinct danger when administered with chenopodium. Dr. Stiles related that he had repeatedly taken the drug personally, during lectures or when giving it to patients, and that on only one occasion had he felt ill effects; on this occasion, a 3 c.c. dose had been followed with unpleasant intoxication-like effects which lasted nearly 36 hours.

The following manuscript was presented by Miss Cram:

Nematodirus furcatus a Synonym of *N. filicollis*, by H. G. May

Soon after the publication of my paper on "Observations on the Nematode genus Nematodirus with descriptions of new species" (Proc. U. S. Nat. Mus.,

58: 577-588) I had the opportunity to talk with Dr. W. L. Chandler, who had submitted the type specimens for my species of *Nematodirus furcatus* to the Bureau of Animal Industry. He expressed the conviction that these specimens were produced by the rupture of specimens of Nematodirus, probably *N. filicollis*. During this process the spicule had in some way been reversed, the tip remaining attached to the body.

More recently Dr. Ransom of the Bureau of Animal Industry kindly offered to send me the type material if I cared to reexamine it to verify Dr. Chandler's interpretation. An examination of this material, however, seemed to give no conclusive evidence to show that the specimens had been ruptured with an extrusion of the posterior end of the spicules. One did show some evidence of a possible rupture.

Since the description of the species was based mostly on the fact that these specimens were much smaller than those of *N. filicollis*, I felt that additional evidence was desirable. Upon request, Dr. Chandler kindly sent me some of his bulk material in lacto-phenol. Although this showed none of the freak forms it did show that the material had suffered severely either before preservation or during the process of preservation, for, although most of the other nematodes were apparently in good condition, the specimens of Nematodirus were mostly not intact. Most of the males had been broken just in front of the spicules. Some very short spicules were also found in this material.

The material showed, further, that there exists in Nematodirus a cuticular thickening around the spicules forming a spicule sheath that extends some distance into the body of the male. It is undoubtedly a part of this sheath that adheres to the point of the spicules shown in my Figure 27 and makes the spicules appear to be fastened to the body. To explain this particular specimen one must also assume that the point of the spicules was broken off in the process of extrusion.

The final evidence to show that *N. furcatus* is no other than an abnormal specimen of *N. filicollis* was obtained when one of the type specimens was further cleared up in lacto-phenol. In this specimen the spicules were reversed and only partly extruded. When it was cleared, it showed distinctly the tip of the spicules of *N. filicollis* inside of the body. The smaller size of the specimens was probably due to the shrinkage which caused the extrusion of the spicules. It is also possible that some of the Michigan specimens are smaller than any observed in the material at hand in Washington.

Dr. Hall presented a mirror device for examining gastro-intestinal contents for parasites. When gastro-intestinal contents are examined in a glass dish for parasites present, the worms frequently go to the bottom by virtue of gravity and their movements. The non-parasitic contents therefore overlie and obscure the parasites and a view from above frequently fails to disclose all or even many of the parasites. Moving the contents in the dish brings some parasites to light, but others move with the contents. Obviously a view of the bottom of the dish will usually show more of what one wishes to see than will a view from above. Since it is neither safe nor desirable to hold the dish above the eyes, the use of a mirror has been tried as a means of seeing the bottom of the dish. The dish is placed on an iron tripod with a ring-shaped top (Fig. A) and a mirror of suitable size rested against the bottom of the two front legs and against the top of the back leg. In the few trials which have been made with this apparatus it has promised to be of some use. One can examine the top or bottom of the dish in comfort and with a little practice it is easy to remove parasites with forceps, guided by the view in the mirror. (See Fig. A, next page.)

Dr. Hackett related some of his recent experiences, helminthological and otherwise, in South America. In some of his examinations of Paraguayans he found as high as 79 per cent. infection with *Necator americanus*, 65 per cent. with *Trichuris trichiura*, and only 4 per cent. with *Ascaris lumbricoides*. He mentioned the remarkable effects of the war of 1870 on the population of

Paraguay, which reduced the male population of 300,000 to 25,000, with the result of transferring labor usually performed by men to the shoulders of the women. Most of the men at present are under 50 years of age.

C. W. Stiles, *Secretary.*

The seventieth meeting was held Oct. 1, 1923. Sir Arnold Theiler, the guest of the evening, was elected a foreign corresponding member of the society. He gave a talk, illustrated with lantern slides, on

Diseases due to parasites or conveyed by parasites.

Some of the points covered were as follows:

In Africa the incidence and importance of parasites is intimately connected with the annual and geographical distribution of rainfall.

Fig. A

The term "nagaña" has been applied in literature to the disease caused by *Trypanosoma brucei,* but as a native word it is applied to the more prevalent conditions caused by *Tr. congolense* and *Tr. vivax.* After the rinderpest outbreak of 1899, with its destruction to certain game, the trypanosome troubles became much less frequent, but under game protection the game came back and with the settling of new game areas trypanosomes caused much trouble. Certain methods have been found very satisfactory in the treatment of some trypanosome diseases. Tartar emetic intravenously cured cases of infection with *Tr. congolense;* Bayer 205 was good for *Tr. brucei* and *Tr. vivax.* The trypanosomes are carried by the tsetse fly, *Glossina pallidipes,* the distribution of the fly following that of certain game, and naturally of certain requirements of game in the form of vegetation and water. Buffalo, zebra, and the warthog are carriers of the trypanosomes, and the zebra is not shot, as a rule, being rated as inedible and not game. Certain tree clusters and a loamy mold soil favor the occurrence of Glossina. The distribution of the fly is notably and sharply limited. In the rinderpest outbreak previously referred to, the tsetse fly disappeared over large areas, although certain antelope carriers

of nagaña survived the rinderpest outbreak. *Trypanosoma theileri* is not known to be pathogenic as a rule but it seems likely that when it is present in enormous numbers, as is sometimes the case, it is pathogenic.

In former times gross tick infestations were much more common and severe than is now the case, though tick worry is recognized and combated. Lambs are sometimes killed as a result of tick paralysis, due to the bite of an individual tick of the species *Ixodes pilosus,* but the farmers in general have learned to look for the tick and remove it. This tick lives as an adult on sheep, the early stages occurring on small animals. Valley pastures appear safer and mountain pastures more dangerous, so far as infestation with this tick is concerned, the tick being limited to relatively cold areas.

Rhipicephalus appendiculatus is the most important tick. It carries East Coast fever. It changes hosts each molt and gets the parasite of East Coast fever as a seed tick and conveys it as a nymph, or as a nymph and conveys it as an adult, but does not carry it through the egg as does the Texas fever tick of the United States. The parasite *Babesia bigemina* was imported from Madagascar to South Africa in the 70's and spread until it reached the dry country where the tick carriers do not exist. *Boophilus decoloratus* is the most important carrier, but all Rhipicephalids are carriers. *Hyalomma aegypticum* is apparently not a carrier.

Anaplasma marginale was described by Smith and Kilborne under the name of marginal points. Smith, according to Dr. Theiler, now regards these as parasites. The parasite is carried by *Boophilus annulatus* in North America and by *B. decoloratus* in South Africa. Anaplasmosis is known as gall sickness. In a mixed infection with Babesia and Anaplasma, if the infected blood is kept for 8 days at ordinary temperatures Babesia dies out and Anaplasma persists and will be transmitted on injection into a susceptible animal. Ticks from the animals infested with both parasites convey one of them when removed at the crisis of the disease due to that parasite and the other when removed at the crisis of the disease due to the other parasite, these crises occurring at different periods. Babesia causes a hemaglobinuria; Anaplasma does not. *Anaplasma marginale* is regularly found on the margin of the red cell. However, one form is found in the middle of the cell, and this form never kills, although *A. marginale* kills 60 per cent. of cattle on injection. The form occurring in the middle is now used to immunize against the marginal form, with excellent results.

Babesia canis is carried by Haemaphysalis, a three-host tick. The infection is carried from the egg to the adult stage, but is not conveyed by the intermediate stages on intermediate hosts. *Babesia caballi* and *Nuttallia equi* give the same clinical picture, but although the disease due to *B. caballi* can be cured by trypanblue, that due to *N. equi* can not. They are carried by *Rhipicephalus evertsi,* a two-host tick, and are transferred from the nymph to the adult.

East Coast fever is due to *Theileria parva.* The red cells are infected, but the true site of the disease is in the lymphatics. The tick is only infective between the 60th and the 120th hour after it bites; not before or after. Hence, if the tick is promptly killed after it attaches, no disease results. Short interval dipping to destroy ticks, as they attach, quickly stops the disease. The disease may be diagnosed by the swollen external lymph glands, as in the neck and flank. Smears obtained from these afford means of definite diagnosis. The kidneys show areas of proliferating endothelial cells with the agametic forms of *T. parva.* Tumors form in the lymph glands. Brumpt has recently adduced evidence to the effect that *Gonderia mutans* is a true Theileria. On this evidence Theiler believes that Brumpt was dealing with a true Theileria, but not with a form identical with *G. mutans.* In conversation with Theiler, Brumpt is inclined to agree that the form he had was not

G. mutans but the Russian *Theileria annulata.* There is also a Theileria in the bushbok.

Heartwater in sheep and goats is due to an invisible and filterable virus. The pericardium is enlarged and the lungs edematous. In cattle, hemorrhages occur in the intestine. The disease is carried by *Amblyomma hebraeum,* a three-host tick, when in the nymphal or adult stage. The ticks can feed on insusceptible animals and still convey the disease subsequently to susceptible animals. The organism of East Coast fever is lost in such feedings. *A. hebraeum* lives only in certain regions of abundant vegetation. *A. variegatum* also carries this parasite.

Spirochaeta theileri causes a high fever, 106° F., in cattle, with no other symptom. Ticks sent from South Africa to France conveyed this parasite and also that of red-water. One tick may carry four different parasites at the same time, making life history work difficult. Dr. Theiler suggested that heart-water might be due to a *Rickettsia.* In South Africa it is necessary to use short-interval dipping for ticks. Infested places are quarantined for 15 months and the animals dipped every 3 to 4 days for the control of East Coast fever and other tick-borne diseases.

South Africa horse sickness is evidently carried by mosquitoes. In the form known as dunkop, there is dyspnoea with an edema of the lungs so excessive that fluid pours from the nostrils. In the form known as dikkop, from the swelling of the temporal fossae, there are heart lesions with a pronounced myocarditis. The dunkop animal dies in the crisis of the fever, the dikkop animal in the lysis. The disease follows the rainy periods. Horses placed in insect-proof cages at this time survive, where those exposed to mosquito bites die. Animals which recover are immune and their serum can be used to protect non-immune animals, such animals being injected with this serum and with infective serum. The disease kills 95 per cent. of horses attacked by it, 80 per cent. of mules, and does not kill donkeys. Mules are used for making immune serum. Horse serum can be used with a strain killing 60 per cent. of horses. Mosquito-proof stables, smudges, and dips of arsenic, kerosene and soap, as repellents are all protective measures, but the dips must be used twice a week.

Blue tongue is also carried by mosquitoes and is due to an ultravisible, filterable virus. After it has passed through many sheep it becomes fixed and is not fatal. This fact is taken advantage of to secure a protective serum. Sheep may be dipped as a protective measure at short intervals in a carbolic dip, but this is not good for the wool. A Leucocytozoon is found in the blood of the ostrich. The carrier of this not known, but a hippoboscid is suspected of playing this role.

For stomach worms in sheep a mixture of copper sulphate and sodium arsenite is used, the first treatment involving two dosings on successive days and the subsequent treatments being single doses at monthly intervals, until the last treatment, which is again two doses on successive days. The drugs are given as a powder and dosing spoons are supplied with the powder. It was found that where farmers gave the copper sulphate solution to flocks, which in South Africa average 2000 to 3000 sheep, drenching accidents were common, the drug entering the lungs. The powder treatment has been given since 1916 to 60 to 80 million sheep. The cost is low, about 1 shilling for 100 sheep.

Dr. Theiler reported some very interesting observations in connection with nodular worm disease in sheep and lambs, not yet published. He also noted a correlation between the character of the vegetation and the prevalence of worm diseases in sheep, and a greater susceptibility to injury by stomach worms on the part of the African and Persian sheep as compared with the wool sheep. The ostrich is infested with a Trichostrongylus which enters the glands of the stomach and causes serious trouble. *Stilesia hepatica,* originally

a parasite of the antelope, occurs in the liver of sheep and goats and occasionally in cattle. *Davainea struthionis* occurs in the ostrich. Turpentine has been found of value as an anthelmintic for the control of this parasite. *Cysticercus cellulosae* occurs in the pig. Dr. Theiler showed a picture of a severe case of cerebral cysticercosis due to this parasite in man, the case following auto-infection.

In work on sheep scab it was found that the life cycle of the scab parasite required 9 days and not 14 as is often stated. Dipping should therefore be repeated in 10 days. The egg was found to survive only 6 to 8 days, and would certainly be dead in 13 to 14 days. Dr. Theiler detailed experiments in which attempts to infect sheep with scab by exposure to infected premises containing infected wool failed.

Dr. Mohler noted that live stock from South Africa could now enter the United States for the first time, due largely to the work of Dr. Theiler. Dr. Ransom noted that *Trypanosoma americanum* Crawley and various other species of non-pathogenic trypanosomes reported from different parts of the world were very similar to *Tr. theileri,* though they were very scarce in individual animals and had not been reported in the heavy infestations reported by Dr. Theiler for *Tr. theileri.*

Miss Cram presented the following note sent in by Prof. H. A. Baylis:

Toxascaris leonina as a parasite of the domestic cat

It is now sixteen years since attention was drawn by Leiper to the existence of the two genera Belascaris and Toxascaris among the Ascaridae of carnivores. While it is well known that a species of each occurs commonly in the dog, records of ascarids found in the domestic cat seem to refer only to *Belascaris mystax.*

Toxascaris leonina (v. Linstow, 1902) is of common occurrence in the lion, tiger, leopard and other Old-world Felidae, both in a wild state and in captivity, but does not seem to have been recognized as a parasite of the cat. It is perhaps of some interest, therefore, to record the fact that on two occasions lately specimens believed to belong to this species, from domestic cats in England, have been added to the helminthological collection of the British Museum. In the first case the material consisted of a single female specimen labeled "cat, London," without further details of its history. In the second, examples of both sexes were collected together with *Belascaris mystax,* from a cat at Cambridge.

Dr. Cobb presented a note with illustration on jointed setae of nematodes. These jointed setae usually have the contour of an extended telescope, but in a species recently observed the joints are insected like the legs of insects. These setae are movable by means of contractile tissue attached at the basal portion. Some setae have terminal organs which are evidently not tactile in function.

The following note presented by Miss Cram at the sixty-second meeting was omitted from the published proceedings:

Hymenolepis tenuirostris, the apparent cause of losses among American geese

Specimens of a tapeworm believed to be responsible for heavy losses among geese in a flock in Oregon were sent in by Dr. J. N. Shaw of Corvallis in July, 1921. The birds showed weakness, emaciation, incoordination and diarrhea. The worms were identified in the Zoological Division of the Bureau of Animal Industry as *Hymenolepis tenuirostris.* This is the first record of this tapeworm from the United States. It has been previously reported from various parts of Europe (England, France, Germany, Denmark, Bohemia, Galicia and Spitzburg).

M. C. Hall, *Acting Secretary.*

BOOK REVIEWS

DISEASE IN CAPTIVE WILD MAMMALS AND BIRDS. By Herbert Fox. J. B. Lippincott Company, Philadelphia, 1923. 673 pp. 87 figures.

This impressive volume is undoubtedly one of the most extensive and significant contributions to knowledge of the subject which has yet appeared in any language. It is based upon material obtained at the Philadelphia Zoological Garden and represents collected studies of the past twenty years. The text is in every way worthy of the splendid setting which has been given to it by the publishers and the size and appearance of the volume make it conspicuous among contributions not only in this special field but even in a wider circle of allied studies. The reviewer can find no record of any single work embracing anything like as much descriptive and comparative material on animal pathology. The studies in this line embrace the major part of the volume and are classified in accordance with the system or organ of the body which is involved. The treatment is exceedingly broad and involves the comparison and contrast of the conditions found with those of a similar character known in the human species. The author has included data on the management of communicable diseases and separate chapters on the relation of diet to disease by E. P. Corson-White and on animal parasites by F. D. Weidman. There is also a very interesting introduction by Charles B. Penrose, President of the Zoological Society of Philadelphia. This reviews the range of the study, the estimate of its worth by the staff of the Philadelphia Zoological Garden and an interesting comparison between conditions in such animals in the wild state and those that are kept in captivity.

The figures are entirely new and in many cases very striking. They are printed on plate paper as inserts and constitute an extremely valuable feature of the work. In addition numerous temperature charts are printed as text inserts.

It would be inappropriate for the reviewer and for the Journal to discuss in detail the features of the book which belong to pathology but a few words regarding the section on animal parasites may be in place. The discussion in this chapter is of a general character and exact data are in the main lacking. While the discussion is interesting it is unfortunate that sufficient attention was not devoted to the subject to work out in detail the parasites concerned. Such a range of observations and mass of material has hardly been at the disposal of any investigator in the field previously. Its complete treatment would have furnished a work of extraordinary value for all desiring information on this topic. As it stands the chapter is rather a discussion under headings of general interest in parasitology than a particular treatment of the material obtained from the Philadelphia Zoological Garden. Indeed the conclusions drawn are general in type and the illustrations employed are cited from other authors or discussed in general terms rather than on the basis of the precise information concerning the causal organism that one would like to get. Evidently, however, it would have been difficult within the limit of less than 50 pages devoted to this phase of the question to have discussed more than these general features. They are of course in no sense unique for they represent questions that have been discussed by parasitologists for many years. There are however valuable new instances of the principles cited and interesting illustrations of the conditions found in specific cases of parasitic infection.

One cannot help noting the way in which the author has departed from rule and usage in printing technical generic names without an initial capital and even extending the same practice to family names. There is, however,

no consistency in the book as many scientific names, including especially those of hosts, are printed in the usual fashion. Misprints are not usual although where they occur in proper names (e. g. Hassal for Hassall) they are conspicuous and unfortunate. The same may be said of such words as *Endameba* where the simplification of the diphthong cannot be defended.

As the subject of animal parasites in its proper extension is no doubt too large to be treated in a single chapter in such a work it is to be hoped that the extremely valuable material which has been collected may be subjected to further study. It would be ideal if on the basis of such study that portion of the subject might be treated in a companion volume as attractive and well worked out as is this treatise.

PARASSITOLOGIA ANIMALE. By DAVIDE CARAZZI. Società Editrice Libraria, Milano, 1922. 467 pp., 236 figures. 4 plates.

The second edition of this well known work requires more than mere superficial mention. The changes made are good and have expanded the volume to nearly 500 pages so that in fact the volume may be regarded rightly as a new book. The author considers each of the main subdivisions of the animal kingdom following in his work the zoological order and taking up under each group its general morphology and physiology as an introduction to the consideration of individual species. On the whole he has maintained a judicious balance in his treatment of the different topics. In accord with the large amount of new material in the field the section on protozoa has been most considerably expanded and next to it the section on arthropods.

The text is especially well written and the selection of the material is on the whole good. In the main the citations are accurate but one notices that numerous figures are credited to some general text from which they have been taken rather than to the original authors responsible for them. One must regret the absence of all references to sources of information, even though the author confesses that this is intentional. The typography of the work is admirable and the illustrations both good and numerous. There are indeed four colored plates in addition to the 235 text figures. Almost all of the latter are new and the exceptions are good. There is a fortunate absence of old rough diagrammatic figures although one or two still remain like that of *Cladorchis watsoni*. The work deserves a high rating and is an important aid to the study of medical zoology not only in Italy but even more widely. One must rejoice to find in a technical treatise such a clear and attractive literary style.

THE REVUE PRATIQUE DES MALADIES DES PAYS CHAUDS (EGYPTE MÉDICO-CHIRURGICALE), began publication in October, 1922.

Its special field and purpose are adequately indicated by the title. Into its service has been drawn an extensive and distinguished series of collaborators, and the articles, many of which naturally deal with topics in Parasitology, represent in the main summaries of present day knowledge. One hardly needs to emphasize the value of such treatment at the hand of a master in the field. Original articles are supplemented by brief news of societies, institutions and special publications in this field.

NOTES

The London School of Tropical Medicine has recently published in its Memoir Series a valuable study on Filariasis in British Guiana which is a report of the Filariasis Commission for 1921. The report includes a careful study of the geography and physiography of the region, of the clinical aspects and pathology of the disease and of its therapy. The Commission reaches a clear conclusion as to the absence of racial immunity and of any specific filariacidal drug. They believe that the total eradication of the disease depends upon prophylaxis which is of course the total elimination of the mosquito carrier from the dwellings of people.

Bronchial spirochetosis in Venezuela is exhaustively treated in a recent monograph by Jesus Rafael Risquez published by the Academia Nacional de Medecina in Caracas.

NECROLOGY

Professor Ludvig von Graff who has suffered for several years from physical breakdown died on February 6 at the age of 74. Among the numerous splendid contributions to biology that came from his pen are several of outstanding character in the field of parasitology. It is fitting to pay this brief tribute to the memory of one whose personality and ability made him one of the leaders in zoology during the period immediately preceding the war.

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